1521-0081/65/4/1162–1197$25.00
PHARMACOLOGICAL REVIEWS

http://dx.doi.org/10.1124/pr.112.007120
Pharmacol Rev 65:1162–1197, October 2013

ASSOCIATE EDITOR: QIANG MA

Therapeutic Targeting of Autophagy in Disease: Biology and Pharmacology

Yan Cheng, Xingcong Ren, William N. Hait, and Jin-Ming Yang

Department of Pharmacology and The Penn State Hershey Cancer Institute, Pennsylvania State University College of Medicine and Milton S. Hershey Medical Center, Hershey, Pennsylvania (Y.C., X.R.,J-M.Y.); and Janssen Research and Development, Raritan, New Jersey (W.N.H.)

This work was supported by the National Institutes of Health National Cancer Institute [Grant R01 CA135038] (to J.Y.); Elsa Pardee Foundation (to J.Y.); and the Department of Defense [Grant BC103654] (to Y.C.).

Address correspondence to: Dr. Jin-Ming Yang, Department of Pharmacology and Penn State Hershey Cancer Institute, Pennsylvania State University College of Medicine and Milton S. Hershey Medical Center, 500 University Drive, Hershey, PA 17033. E-mail: jyang2@hmc.psu.edu

dx.doi.org/10.1124/pr.112.007120.

***Abstract*——Autophagy, a process of self-digestion of the cytoplasm and organelles through which cellular components are recycled for reuse or energy production, is an evolutionarily conserved response to metabolic stress found in eukaryotes from yeast to mammals. It is noteworthy that autophagy is also associated with various pathophysiologic conditions in which this cellular process plays either a cytoprotective or cytopathic role in response to a variety of stresses such as metabolic, inflammatory, neurodegenerative, and therapeutic stress. It is now generally believed that modulating the activity of autophagy through targeting specific regulatory molecules in the autophagy machinery may impact disease processes, thus autophagy may represent a new pharmacologic target for drug development and therapeutic intervention of various human disorders. Induction or inhibition of autophagy using small molecule compounds has shown promise in the treatment of diseases such as cancer. Depending on context, induction or suppression of autophagy may exert therapeutic effects via promoting either cell survival or death, two major events targeted by therapies for various disorders. A better understanding of the biology of autophagy and the pharmacology of autophagy modulators has the potential for facilitating the development of autophagy-based therapeutic interventions for several human diseases.**

I. Introduction: Autophagy as a New Therapeutic Target

Autophagy, literally meaning "self-eating" in Greek and discovered more than half a century ago, is an evolutionarily conserved and genetically controlled cellular response to nutrient deprivation that is found in yeast, plants, worms, flies, mice, and humans. This cellular catabolic process is characterized by formation of double-membrane vesicles ("autophagosomes") in the cytosol that first engulf organelles and cytoplasm and then fuse with the lysosomes to form the autolysosomes in which the contents are degraded and recycled for synthesis of ATP and various macromolecules such as proteins (Levine and Klionsky, 2004). In the absence of stress, basal autophagy may function as a housekeeper by degrading intracellular damaged proteins and organelles (Levine and Klionsky, 2004).

This form of self-digestion in both unicellular and multicellular organisms is commonly activated in times of nutrient deprivation as a temporary cellular protective mechanism leading to self-preservation (Maiuri et al., 2007b). Cellular stress can prompt cells to exit the cell cycle, shrink, autodigest long-lived proteins and damaged organelles through autophagy, and recycle fatty acids and amino acids for either synthesis of macromolecules or oxidation in the mitochondria to maintain cellular ATP. If left unchecked, however, autophagy has the potential of causing terminal self-

ABBREVIATIONS: 1,25D3, 1α,25-dihydroxycholecalciferol; ABT-737, *N*-{4-[4-(4′-chloro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-4-(3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide; AD, Alzheimer disease; AKT, protein kinase B; AMPK, AMP-activated protein kinase; AP-23573, deforolimus; AR-12, 2-amino-*N*-[4-[5-(2 phenanthrenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] phenyl]-acetamide; α-syn, α-synuclein; Atg, autophagy-related genes; AZD8055, 5-[2,4-bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl]-2-methoxyphenyl]methanol; BH3, Bcl-2 homology domain 3; CCI-779, temsirolimus; CMA, chaperone-mediated autophagy; CQ, chloroquine; CR, caloric restriction; DRAM, damage-regulated autophagy modulator; eEF2K, eukaryotic elongation factor-2 kinase; EGFR, epidermal growth factor receptor; FTI-276, (2*S*)-2-[[4-[[(2*R*)-2-azaniumyl-3-sulfanylpropyl]amino]-2-phenylbenzoyl]amino]-4-methylsulfanylbutanoate; HA14-1, 2-amino-6-bromo-α-cyano-3-(ethoxycarbonyl)-4*H*-1-benzopy ran-4-acetic acid ethyl ester; HCQ, hydroxychloroquine; HD, Huntington disease; HSC70, 70-kDa chaperone heat-shock cognate protein; Htt, huntingtin; IFN, interferon; IL, interleukin; IP3, inositol 1,4,5-trisphosphate; IP3R, inositol 1,4,5-trisphosphate receptor; I/R, ischemia/reperfusion; L-690,330, 1-(4-hydroxyphenoxy)-1-phosphonoethyl]phosphonic acid; LAMP, lysosomal-associated membrane protein; LAQ824, (2*E*)-*N*-hydroxy-3-[4-[[(2-hydroxyethyl)[2-(1*H*-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide; LC-3, light chain 3; LKB1, liver kinase B1; LY294002, 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one hydrochloride; 3-MA, 3-methyladenine; MK-2206, 8-[4-(1-aminocyclobutyl)phenyl]-9-phenyl-1,2,4-triazolo[3,4-*f*][1,6]naphthyridin-3(2*H*)-one dihydrochloride; mTOR, mammalian target of rapamycin; mTORC, mTOR signaling complex; NPI-0052, (1*R*,4*R*,5*S*)-4-(2-chloroethyl)-1-((*S*)-((*S*)-cyclohex-2-enyl)(hydroxy)methyl)-5-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione; NVP-BEZ235, 2-methyl-2-{4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl]phenyl}propanenitrile; OSI-906, 3-[8-amino-1-(2-phenylquinolin-7-yl)imidazo[1,5-a]pyrazin-3-yl]-1-methylcyclobutan-1-ol; OSU-HDAC42, AR42, (*S*)-(+)-*N*-hydroxy-4-(3-methyl-2-phenyl-butyrylamino)-benzamide; PD, Parkinson disease; PE, phosphatidylethanolamine; PI-103, 3-4-(4-morpholinylpyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidin-2-yl-phenol; PI3K, phosphatidylinositol-3 kinase; polyQ, polyglutamine; PP242, 2-(4-amino-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1*H*-indol-5-ol; PP30, 3-(4-amino-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-*N*-(4,5-dihydrothiazol-2-yl)benzamide; PTEN, phosphatase and tensin homolog; RAD-001, everolimus; ROS, reactive oxygen species; SAHA, vorinostat; SQSTM1, sequestosome 1; STF-62247, *N*-(3-methylphenyl)-4-pyridin-4-yl-1,3-thiazol-2-amine; SUL, sulfaphenazole; Torin1, 1-[4-(4-propanoylpiperazin-1-yl)-3-(trifluoromethyl)phenyl]-9-quinolin-3-ylbenzo[h][1,6]naphthyridin-2-one; TNF-α, tumor necrosis factor α; TSC, tuberous sclerosis complex; UPS, ubiquitin-proteasome system; ULK1, mammalian homolog of Atg1; UVRAG, UV radiation resistance-associated gene; WYE-125132, 1-[4-[1-(1,4-dioxaspiro[4.5]decan-8-yl)-4-(8-oxa-3-azabicyclo[3.2.1]octan-3-yl)pyrazolo[3,4-d]pyrimidin-6-yl]phenyl]-3-methylurea; z-VAD, *N*-benzyloxycarbonyl-Val-Ala-Asp.

consumption and cannibalistic cell death. Under certain circumstances, autophagy is also considered a form of nonapoptotic cell death termed "type II programmed cell death" or autophagic cell death (Bergmann, 2007). Whereas autophagic cell death rests largely on circumstantial evidence, autophagic cell survival is supported by direct evolutionary, genetic, and biochemical studies. Autophagy is induced by a broad range of intracellular or extracellular stresses to degrade protein aggregates, oxidized lipids, damaged organelles, and even intracellular pathogens, thus having a variety of physiologic and pathophysiologic roles such as development and differentiation, life-span extension, elimination of microorganisms, and antigen presentation (Ravikumar et al., 2010).

In recent years, accumulating evidence has pointed to the importance and functional role of autophagy in various human diseases. For instance, it is becoming increasingly clear that altered autophagy activity is associated with tumor formation and progression as well as with altered response to cancer therapy (Kondo et al., 2005; Janku et al., 2011). Numerous studies indicate that autophagy is activated in tumor cells exposed to certain types of chemotherapy and targeted therapy (Kondo et al., 2005; Janku et al., 2011). Defects in autophagy are found to be involved in premature aging (Rajawat et al., 2009), neurodegenerative diseases, and cardiovascular disorders (Mizushima et al., 2008; Rubinsztein et al., 2012). In addition, it appears that defects in autophagy can lead to an increased susceptibility to infection and can be associated with autoimmune and inflammatory diseases (Deretic and Levine, 2009; Deretic, 2010; Levine et al., 2011).

Recently, substantial progress has been made in identifying genes, proteins, signaling pathways, and related molecular mechanisms that are involved in autophagy regulation. This has permitted the manipulation of autophagy by inhibiting or activating autophagy-related genes and proteins using genetic and/or pharmacologic approaches. Notably, an increasing number of autophagy-related genes, proteins, and pathways have been found to be aberrant or altered in human diseases, offering novel targets for therapeutic intervention. Indeed, modulating autophagic processes has already shown promise in the treatment of certain human diseases, and autophagy has been emerging as a promising and attractive new target of great interest to the pharmaceutical industry. Recently, great efforts have been made in developing autophagy-based pharmacotherapy for various human health problems. This review will provide an overview of our current understanding of the biology of autophagy and the roles that autophagy plays in the pathophysiology of various human disorders. Our goal is to show how autophagy modulators may lead to a new generation of therapeutic agents for the treatment of various human diseases.

II. Cellular and Molecular Biology of Autophagy

A. Types of Autophagy and the Basic Process

Three types of autophagy have been defined based on the process and mechanisms by which the target substrates are delivered to lysosomes for degradation: macroautophagy (Fig. 1A), chaperone-mediated autophagy (CMA) (Fig. 1B), and microautophagy (Fig. 1C).

Macroautophagy (hereafter referred to as autophagy) involves the sequestration of intracellular components (such as proteins and organelles) into newly formed double-membrane vesicles termed autophagosomes, which subsequently fuse with the lysosomes for degradation. Schematically, the process of autophagy can be characterized by three main operational steps (Weidberg et al., 2011). The first is the formation of autophagosomes and sequestration of cytoplasmic material into vesicles. This process involves three stages as follows: 1) initiation, the formation of the phagophore; 2) nucleation, the engulfment of the cytoplasmic material by the phagophore; and 3) elongation/enclosure, the phagophore membrane elongation and fusion of its edges to form the autophagosome. The second is the fusion of the autophagosome with the lysosome to form the autolysosome. The third is the degradation of the sequestered materials for recycling and energy production.

Macroautophagy is further classified into two types as follows: nonselective autophagy and cargo-specific autophagy, which includes mitophagy, pexophagy, ribophagy, and xenophagy (Youle and Narendra, 2011). Mitophagy, which mediates the selective elimination of damaged or excessive mitochondria, plays an essential role in maintaining mitochondrial quantity. Removal of damaged mitochondria through autophagy requires two steps as follows: induction of general autophagy and priming of damaged mitochondria for selective autophagic recognition (Ding and Yin, 2012).

During the process of CMA, cytosolic proteins are recognized by heat-shock cognate protein (HSC70), a 70-kDa chaperone that mediates the translocation of proteins to the surface of the lysosomes where substrates bind to the lysosomal-associated membrane protein type 2A (LAMP-2A), leading to protein internalization and degradation (Kaushik and Cuervo, 2012). CMA selectively recognizes proteins containing a KFERQ motif at the C-terminal sequence. This particular pentapeptide consensus motif is the only binding region recognized specifically by HSC70 for protein delivery to the lysosomes and is necessary and sufficient for degradation of the substrates through CMA (Chiang et al., 1989; Dice, 1990). CMA involves the following steps: 1) recognition of the KFERQ region of cytosolic proteins by HSC70; 2) binding of substrate proteins to the cytosolic tail of LAMP-2A; 3) LAMP-2A multimerization and substrates translocation inside the lysosomes; and 4) substrate degradation and

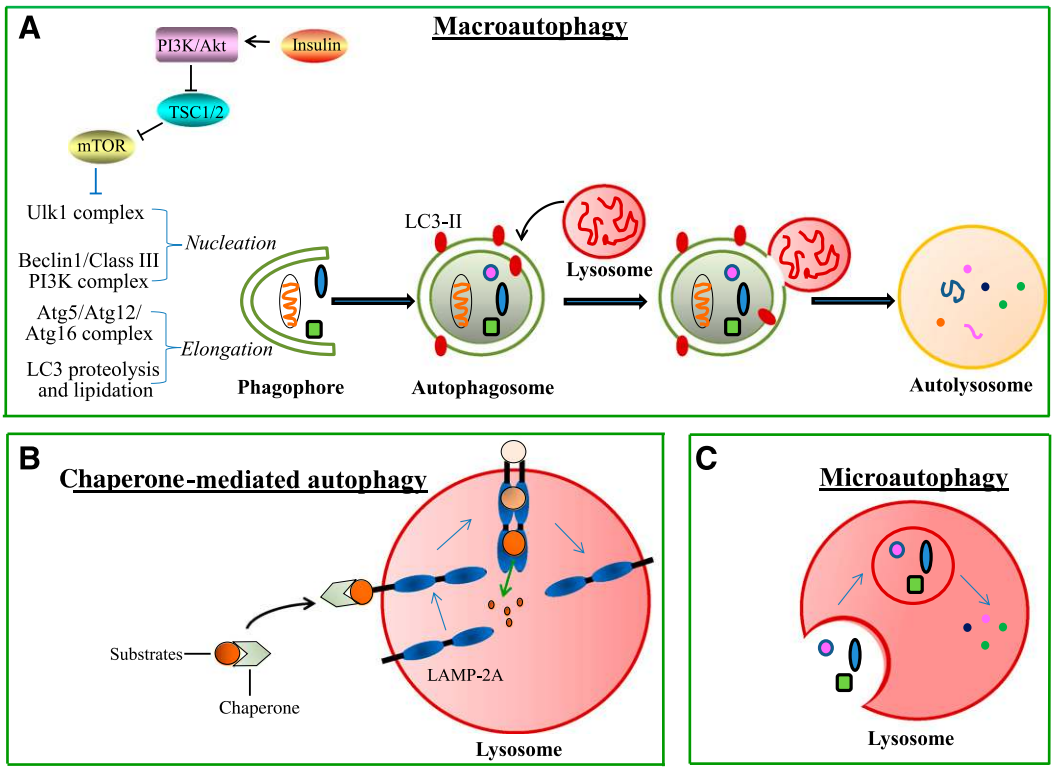


Fig. 1. The processes of autophagy. (A) Macroautophagy. Macroautophagy begins with engulfment of the cytoplasmic materials by the phagophore, which sequesters the materials into a double-membrane vesicle (i.e., autophagosome). The autophagosome fuses with a lysosome to form an autolysosome, and then the cytoplasmic materials are degraded by the lysosome. The initiation step of autophagosome formation requires the ULK1-Atg13-FIP200 complex and Beclin1-class III PI3K complexes. Two conjugation systems, Atg12-Atg5-Atg16 and Atg8-PE, are essential for the elongation and enclosure step of the autophagosome formation. Lipid conjugation leads to the conversion of the soluble form of LC3-I to the autophagic vesicle-associated form LC3-II, which is commonly used as a marker of autophagy. mTOR plays a critical role in regulating autophagy: under nutrient-rich conditions, mTOR is activated and inhibits autophagy through repression of ULK1 activity (the mammalian homologs of ATG1). Growth factors such as insulin or insulin-like growth factor can activate the class I PI3K-Akt/PKB pathway, which phosphorylates tuberous sclerosis complex (TSC2) and prevents the formation of an TSC1/2 protein complex, resulting in activation of mTOR. (B) Chaperone-mediated autophagy. During chaperone-mediated autophagy, the cytosolic proteins bind to the LAMP-2A receptor in an Hsc70 chaperone-dependent manner for translocation to the lysosomes, leading to their internalization and degradation. (C) Microautophagy. Microautophagy involves the direct sequestration of the cellular components by the lysosome through invagination of the lysosomal membranes.

LAMP-2A dissociation into monomers (Chiang et al., 1989; Arias and Cuervo, 2011).

Microautophagy involves direct internalization of cytoplasmic materials into the lysosome. Cellular constituents are sequestrated into the lysosomal lumen for degradation through invagination of the lysosomal membrane, which subsequently pinches off to form small vesicles (Mortimore et al., 1988).

B. Physiologic Role of Autophagy

Autophagy is evolutionarily conserved in eukaryotes from yeast to mammals and has miscellaneous physiologic roles, including adaptation to starvation, clearance of intracellular proteins and organelles, development and differentiation, life-span extension, elimination of micro-organisms, and antigen presentation. The basal level of autophagy is generally viewed as a cellular housekeeper that is crucial for quality control of the integrity of intracellular proteins. In metabolic or other types of stress, induction of autophagy is important for intracellular generation of amino acids and other nutrient molecules for cellular survival. Conversely, excessive autophagy can cause nonapoptotic programmed cell death.

1. Autophagy-Dependent Cellular Homeostasis. There are two known cellular mechanisms for protein degradation: the ubiquitin-proteasome system (UPS) and the autophagy-lysosome system. The UPS is the major pathway for degradation of short-lived proteins (Hershko and Ciechanover, 1998), whereas the autophagy is mainly responsible for degradation of long-lived proteins and other cellular contents and is the mechanism by which the large structures such as organelles and protein aggregates are degraded (Rabinowitz and White, 2010). Under normal conditions, low level, constitutive autophagy participates in maintaining cellular homeostasis by degrading and recycling unnecessary or excessive cellular components, eliminating abnormal, aged, or potentially harmful molecules and organelles, and removing intracellular pathogens (Levine, 2005).

Impaired autophagy may result in accumulation of abnormal and misfolded protein aggregates. In neural cells and hepatocytes from mice with knockout of the autophagy gene *Atg5*, abnormal intracellular proteins

accumulate and form aggregates and inclusions (Hara et al., 2006). Likewise, mice with deficiency of the autophagy gene *Atg7* accumulate intracellular ubiquitinated aggregates in the cytoplasm of hepatocytes and neurons (Komatsu et al., 2005, 2006). These observations indicate that continuous clearance of the diffused cytosolic proteins through basal autophagy is essential for homeostasis and that autophagy plays a key role in quality control of the cellular components by removing abnormal and potentially harmful proteins.

Autophagy is also involved in monitoring the quality of important organelles such as the mitochondria. Mice with *Atg7* deficiency show multiple cellular abnormalities, including the appearance of concentric membranous structure and deformed mitochondria (Komatsu et al., 2005). The hearts of the *Atg5*-deficient mice display disorganized sarcomere structure, and mitochondrial misalignment and aggregation (Nakai et al., 2007). Mitochondria with loss of integrity can be selectively removed by autophagy, a process termed "mitophagy" (Marino and Lopez-Otin, 2004). In rat hepatocytes, nutrient deprivation initiates mitochondrial depolarization and subsequent sequestration of the mitochondria into autophagosomes (Elmore et al., 2001). Although selective degradation of the mitochondria by mitophagy is vital for cellular homeostasis, the molecular mechanisms of mitophagy remain incompletely understood. So far, Atg11, Atg32, and Atg8 have been identified as critical regulators of mitophagy (Kanki and Klionsky, 2008; Kanki et al., 2009; Okamoto et al., 2009). In mammals, autophagic degradation of depolarized mitochondria is initiated by the Pink1-dependent mitochondrial translocation of Parkin, followed by ubiquitination of mitochondrial proteins and recruitment of p62 to direct the mitochondria to autophagosomes (Geisler et al., 2010). Recently, Bif-1, a member of the membrane curvature-driving endophilin family of proteins, was found to be required for autophagy-dependent clearance of damaged mitochondria (Takahashi et al., 2013).

Autophagy also plays critical roles in development and differentiation. For instance, autophagy is vital for the preimplantation development period after oocyte fertilization, and for the early postnatal period during neonatal starvation (Mizushima and Levine, 2010). Altered autophagy can affect embryogenesis, including midembryonic development. *Atg5*, a key autophagy-regulatory gene, is required for the engulfment of apoptotic cells during the morphogenesis of specific organs in late embryonic development (Qu et al., 2007). Autophagy enables cells to undergo structural remodeling and contributes to cellular differentiation during erythropoiesis, lymphopoiesis, and adipogenesis (Rubinsztein et al., 2012).

2. Autophagy-Dependent Cellular Survival. With different types of stress (e.g., such as nutrient deprivation, growth factor depletion, hypoxia, cytotoxic insult), activation of autophagy can generate intracellular metabolic substrates for maintenance of cellular energy demands. The amino acids and fatty acids generated from autophagic degradation of membrane proteins and lipids may provide essential nutrients for survival of the organism subjected to a variety of stresses (Rabinowitz and White, 2010). One of the typical triggers of autophagy is nutrient starvation, which can induce autophagy in yeast, plant cells, mammalian cells, and whole animals to provide nutrients and energy to support survival. Autophagy-defective cells fail to maintain physiologic levels of amino acid; when subjected to starvation, both intracellular and extracellular concentrations of amino acids decrease in autophagy-deficient cells and animals to a greater extent than in wild-type littermates. In yeast cells harboring the *Atg7* mutant, the total pool of intracellular amino acids was reduced, but the wild-type cells maintained amino acid concentrations compatible with life (Onodera and Ohsumi, 2005). *Atg5*-deficient neonatal mice had decreased amino acid concentrations in both plasma and tissues (Kuma et al., 2004). $Atg7^{-/-}$ mice not only had a lower level of amino acids but also died earlier after birth as compared with the wild-type animals (Komatsu et al., 2005). The amino acids and fatty acids generated through autophagy can be used by the tricarboxylic acid (TCA) cycle to maintain ATP production in the mitochondria (Levine and Kroemer, 2008). In addition, those amino acids can be used for protein synthesis, as the impaired protein synthesis in the *Atg7* mutant yeast cells was much more severe than in the wild-type cells during starvation (Onodera and Ohsumi, 2005).

In yeast, autophagy-defective cells are susceptible to starvation-induced damage. Upon nitrogen starvation, plants with mutant autophagy genes show defects in development, including accelerated senescence and enhanced chlorosis (Hanaoka et al., 2002; Yoshimoto et al., 2004). The postnatal survival time of the starved mice deficient for *Atg5* was less than 12 hours but was prolonged by forced milk feeding, indicating the importance of the nutrient supply derived from neonatal autophagy in supporting survival of life (Kuma et al., 2004). Similarly, mice deficient in *Atg7* suffered from severe nutrient and energy insufficiency shortly after birth (Komatsu et al., 2005).

Equally important, cellular autophagy also plays a cytoprotective or prosurvival role under stressful conditions such as growth factor deprivation (Lum et al., 2005) and treatment with chemotherapy or anti-infective agents (Maiuri et al., 2007b). These studies provide compelling evidence that the nutrients generated through autophagy are important for survival during stress, supporting the notion that autophagy is a self-protective mechanism of living organisms under various stressful conditions.

3. Autophagic Cell Death. Above and beyond the studies of the role of autophagy in cellular survival,

there has also been extensive interest in autophagy-associated cell death, referred to as type II programmed cell death. As early as the 1960s, autophagic vacuoles containing organelles such as mitochondria were first observed in dying animal cells (Ashford and Porter, 1962). This area of investigation is confounded by the possibility that the appearance of autophagy in dead or dying cells is an unsuccessful cellular attempt at survival. As mentioned previously, cells or organisms deficient in autophagy may die more rapidly rather than more slowly. Subsequent studies found that autophagic cell death often occurs when groups of cells or entire tissues die during development, and these dying cells contain "autophagic vacuoles" in the cytoplasm (Baehrecke, 2002). More recently, autophagic cell death was observed in tumor cells treated with chemopreventive or chemotherapeutic agents such as resveratrol (Opipari et al., 2004), arsenic trioxide (Kanzawa et al., 2003), and tamoxifen (Bursch et al., 1996). Support for the role of autophagy in cell death was provided via the use of autophagy inhibitors, which can rescue cell death resulting from autophagy. For example, 3-methyladenine (3-MA), an inhibitor of class III phosphatidylinositol-3 kinase (PI3K) that blocks the initial step of autophagy, has been reported to suppress autophagy and inhibit cell death in several cell lines subjected to various drug treatments including tamoxifen (Bursch et al., 1996), tumor necrosis factor α (TNF-α) and bacterial toxins (Bursch, 2001). Studies also showed that knockdown of some key autophagy genes can reduce cell death. The small molecule compound STF-62247 [*N*-(3-methylphenyl)-4-pyridin-4-yl-1,3-thiazol-2-amine] promotes cell death in renal cell carcinoma both in vitro and in vivo through inducing autophagy, and inhibition of autophagy by the small-interfering RNA (siRNA) against *Atg7* or *Atg9* decreases the sensitivity of the tumor cells to STF-62247 (Turcotte et al., 2008). Nonapoptotic cell death induced by z-VAD, a caspase inhibitor, was decreased when cells were cotreated either with the autophagy inhibitors 3-MA and wortmannin or subjected to the RNAi-mediated knockdown of *Atg7* and *Beclin1;* this suggests that inhibition of caspase arrests apoptosis but activates autophagic cell death (Yu et al., 2004). In $Bax^{-/-}Bak^{-/-}$ murine embryonic fibroblasts (MEFs) treated with etoposide (a topoisomerase II poison) or staurosporine (a prototypical ATP-competitive kinase inhibitor), two inducers of apoptosis, nonapoptotic cell death accompanied by autophagic vacuolization was reduced when *Atg5* or *Beclin1* was knocked down (Shimizu et al., 2004). Autophagic cell death can be revealed when apoptosis is blocked (Maiuri et al., 2007b). Additionally, excessive autophagy can cause cannibalistic cell death (Bergmann, 2007). Although there are numerous scenarios in which autophagy-associated cell death may occur, the importance and significance of autophagy in promoting cell death and its relationship with apoptotic cell death are still the subject of intensive investigation (Kroemer and Levine, 2008).

As there are a number of examples of cell death associated with autophagy, the question of how important the autophagic process is in cell death was recently reviewed by Kroemer and Levine (2008). Certain studies suggest that inhibition of autophagy may result in either delayed or partial inhibition rather than prevention of cell death. Because inhibition of autophagy by pharmacologic agents or genetic manipulations also may have some off-target effects on cellular functions, the question of whether cell death can truly occur due to autophagy alone remains to be clarified.

C. Regulation of Autophagy and the Signaling Pathways

The past decade has seen remarkable elucidation of many molecular mechanisms and pathways that are critically involved in the regulation of autophagy. We will now review the studies that have facilitated our understanding of the implications of autophagy in human health and disease.

1. Molecular Regulation of Autophagy. Studies of the genes and gene products involved in the regulation of autophagy have advanced our knowledge of the molecular basis of this cellular process. To date, more than 30 autophagy-related genes (Atg) have been identified in yeast, most of which function in the formation of autophagosome (Yang and Klionsky, 2010). All the Atg proteins found in yeast have mammalian homologs, suggesting that the molecular machineries of autophagy operating in yeast are also used in other eukaryotic cells.

Beclin1, the mammalian homolog of yeast autophagy gene *Atg6*, was the first identified autophagy gene in mammalian cells (Li et al., 2012). The initiation step of autophagosome formation requires the Beclin1-class III PI3K complex, which contains Beclin1, Vps34 (class III PI3K), and p150 (Simonsen and Tooze, 2009). Recently, Beclin1 was found to interact with additional proteins, including Atg14, UV radiation resistance-associated gene (UVRAG) product (Liang et al., 2006; Itakura et al., 2008; Li et al., 2012), and activating molecule in Beclin1-regulated autophagy protein 1 (AMBRA1) (Fimia et al., 2007). All these proteins are present in the Vps34 complex and act as inducers of autophagy. The Rubicon protein is a negative regulator of autophagy because when it binds to Beclin1 it reduces the activity of Vps34 and impairs the formation of autophagosome (Zhong et al., 2009). Atg1 plays a key role in the early stage of autophagy by forming a complex with Atg13 and Atg17, both of which enhance Atg1 kinase activity and promote autophagosome generation (Ganley et al., 2009; Hara and Mizushima, 2009; Hosokawa et al., 2009). The components

of this complex are mostly conserved from yeast to human; the serine/threonine kinase ULK1, mATG13, and FIP200 are the mammalian functional homologs of the yeast Atg1, Atg13, and Atg17, respectively. Atg13 promotes the formation of the Atg1-Atg1 self-interaction complex, which is correlated with the kinase activity of Atg1 and the amount of autophagy (Yeh et al., 2011). Atg8 was recently identified as a regulator of Atg1, which directly binds to Atg1 and enhances its activity in autophagy (Kraft et al., 2012).

Two ubiquitin-like conjugation systems, the Atg12-Atg5-Atg16 complex and the Atg8-phosphatidylethanolamine complex (PE), participate in the elongation/enclosure step that is critical for autophagosome formation. These conjugation systems are conserved in various eukaryotes. In the first ubiquitin-like system, Atg12 is conjugated to Atg5 through Atg7 and Atg10, an E1-like ubiquitin-activating enzyme and an E2-like ubiquitin-conjugating enzyme, respectively (Mizushima et al., 1998). The E1-like Atg7 binds to the C-terminal glycine of Atg12 through its active cysteine site to form an intermediate complex via a thioester bond. Subsequently, Atg12 is activated by ATP hydrolysis and transferred to the E2-like Atg10. The C-terminal glycine of Atg12 then covalently binds to an internal lysine residue of Atg5 to form the final conjugate. The Atg5-Atg12 conjugates interact noncovalently with Atg16 (Mizushima et al., 1999). The Atg12-Atg5-Atg16 complex then associates with phagophores and dissociates when formation of autophagosomes is completed (Yorimitsu and Klionsky, 2005).

The second conjugation system involves the cleavage and lipidation of Atg8 (Ichimura et al., 2000). Atg8 is cleaved near the C-terminal glycine residue by the cysteine protease Atg4, subsequently is activated by the E1-like Atg7, is transferred to the E2-like Atg3, and eventually is conjugated to the lipid PE. Atg8-PE can be detected on both of the intermediate vesicle and the completed autophagosome. This complex is transported to the lysosome/vacuole and degraded along with the cargo. There are at least three Atg8 homologs in mammalian cells: GATE-16 (Golgi-associated ATPase enhancer of 16 kDa), GABARAP (γ-aminobutyric acid type A receptor-associated protein), and MAP1LC3 (microtubule-associated protein 1 light chain 3). All these proteins are modified with lipid in the same manner as occurs in yeast, and all localize to the autophagosome (Kabeya et al., 2004). In mammalian cells, light chain 3 (LC3) is cleaved by Atg4 to form the cytosolic soluble LC3-I, which can be further cleaved and converted to a membrane-associated form LC3-II through conjugating with the lipid PE (Kabeya et al., 2000).

LC3-II, the cleaved product of LC3-I, is inserted within the inner and outer membrane of the vesicles during autophagosome formation. LC3 has been intensely characterized and, like yeast Atg8, is commonly used as a marker for the mammalian autophagosome. In addition, LC3-II remains on the mature autophagosomes until fusion with lysosomes is completed. Thus, the fusion of the autophagosome with lysosomes can be assessed by colabeling LC3 and LAMP1, a lysosome-associated membrane protein (Weidberg et al., 2011). p62/SQSTM1 (sequestosome 1), an ubiquitin-binding scaffold protein, binds directly to LC3 via a specific sequence motif and is degraded by the autophagic process. p62/SQSTM1 can also link ubiquitinated proteins to the autophagic machinery to enable their degradation in the lysosome (Pankiv et al., 2007). When autophagy is suppressed, p62/SQSTM1 markedly accumulates. In contrast, the amount of p62/SQSTM1 decreases when autophagy is activated, thus p62 is often used as a marker for autophagic flux. Another commonly used method for measurement of autophagy flux is to monitor the conversion of LC3-I to LC3-II in the presence of lysosomal cysteine and/or aspartic proteinase inhibitors such as E-64 day and pepstatin A (Klionsky et al., 2008).

2. Signaling Pathways Regulating Autophagy.

a. Mammalian target of rapamycin. Mammalian target of rapamycin (mTOR), a PI3K-related serine/threonine protein kinase, is a key regulator of cell growth and proliferation. mTOR regulates various cellular functions such as energy metabolism, translation initiation, and cytoskeletal organization (Schmelzle and Hall, 2000). Recently, it was found that the mTOR signaling pathway also may regulate autophagy (Fig. 2). When activated by nutrients, growth factors, or hormones such as insulin, mTOR inhibits autophagy. mTOR is present in two structurally and functionally distinct protein complexes known as mTORC1 and mTORC2. mTORC1 consists of mTOR, mLST8 (mammalian lethal with SEC13 protein 8), Raptor (regulatory-associated protein of mTOR), PRAS40 (proline-rich Akt substrate of 40 kDa), and DEPTOR (DEP domain containing mTOR-interacting protein), and is rapamycin sensitive. mTORC2 contains mTOR, mLST8, Rictor (rapamycin-insensitive companion of mTORC2), mSin1 (mammalian stress-activated MAP kinase-interacting protein 1), and Protor-1/Protor-2 (protein observed with Rictor-1/protein observed with Rictor-2) (Sabatini, 2006; Pearce et al., 2011). mTORC1 is downstream of PI3K and is activated in response to mitogenic stimuli or nutrient availability (Hay and Sonenberg, 2004). Growth factors such as insulin and insulin-like growth factor bind to their receptor tyrosine kinases, leading to autophosphorylation of these receptors, with subsequent phosphorylation of insulin receptor substrates (IRS). Phosphorylated insulin receptor substrates further recruit and activate the class I PI3K-Akt/PKB, which then phosphorylates tuberous sclerosis complex (TSC2) and prevents the formation and ability of TSC1/2 to act as a GTPase-activating protein for Rheb. Rheb activates mTOR1 when bound to GTP. Inhibition of

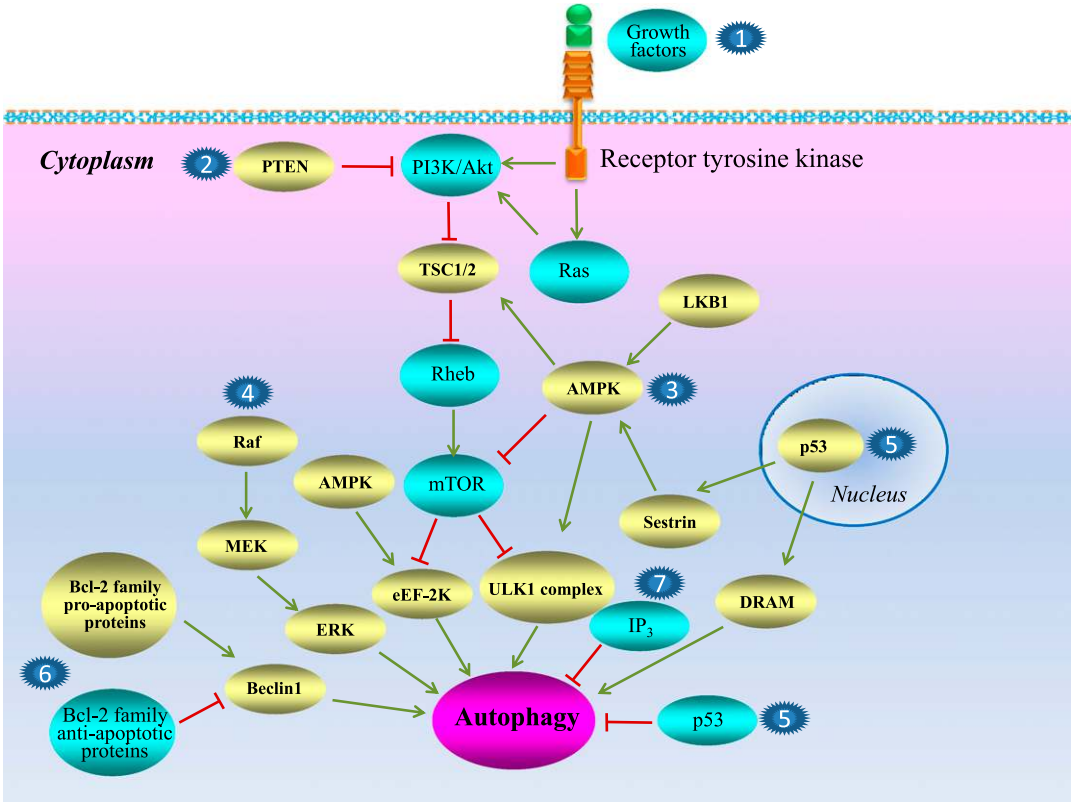


Fig. 2. The signaling pathways involved in autophagy regulation. Yellow ovals: autophagy stimulatory. Blue ovals: autophagy inhibitory. (1) Growth factors bind to their receptor, and activation of the receptor tyrosine kinase stimulates PI3K/Akt and Ras. Akt phosphorylates and inhibits the TSC1/2 complex, and dampens the inhibitory effect of TSC1/2 on Rheb, leading to mTORC1 activation and consequently to autophagy inhibition by affecting ULK1 complex formation. (2) PTEN inhibits PI3K/Akt/mTOR signaling and is an autophagy-promoting signal. (3) AMPK can be phosphorylated and activated by LKB1. Activation of AMPK phosphorylates and activates TSC1/2, leading to inactivation of mTOR and induction of autophagy. AMPK can also cause inactivation of mTOR by directly phosphorylating the mTOR binding partner Raptor. AMPK also regulates ULK1 and coordinates the induction of autophagy. (4) The Raf-1–MEK1/2–ERK1/2 signaling cascade causes the activation of autophagy. (5) Nuclear p53 stimulates autophagy in a transcription-dependent fashion by activating the expression of DRAM and sestrin. By contrast, cytoplasmic p53 is responsible for the inhibition of autophagy. (6) The Bcl-2 family antiapoptotic proteins interact with Beclin1 and exert inhibitory effects on autophagy. Conversely, proapoptotic proteins have stimulatory effects on autophagy by disrupting the association of antiapoptotic proteins with Beclin1. (7) Intracellular IP_3 negatively regulates autophagy via an mTOR-independent mechanism.

TSC1-TSC2 by Akt phosphorylation allows Rheb-GTP to accumulate and activates mTOR (Proud, 2007). Phosphatase and tensin homolog (PTEN), a phosphoinositide-3 phosphatase, antagonizes the signaling of the Akt/PKB pathway and is an activator of autophagy (Shaw and Cantley, 2006). In general, activation of PI3K indirectly regulates AKT (protein kinase B), whose activity prevents tuberous-sclerosis complex-2 (TSC2)-mediated inhibition of Rheb.

Activation of mTOR has been known to be a negative regulator of autophagy. Although the precise mechanism by which mTORC1 controls autophagy and its direct target(s) remains unclear, mTORC1 is believed to act in two ways. First, mTORC1 inhibits the association between Atg1 and Atg13. Under nutrient-rich conditions, the activated mTORC1 induces the phosphorylation of Atg13 and prevents its association with Atg1. When subjected to nutrient deprivation or rapamycin treatment, Atg13 is rapidly dephosphorylated upon mTORC1 inactivation and shows a high affinity for Atg1. Formation of the Atg1-Atg13 complex results in activation of Atg1 and subsequent induction of autophagy (Kamada et al., 2000, 2010). The human homolog of Atg13 is also a direct substrate for mTOR (Jung et al., 2009). Second, mTORC1 inactivates ULK1 by phosphorylating this protein. Inactivation of mTOR1 leads to dephosphorylation and activation of Ulk1 and consequently induces autophagy under conditions of starvation or rapamycin treatment (Kim et al., 2011a; Shang et al., 2011). Our laboratory has been particularly interested in elongation factor 2 kinase (eEF2K), an enzyme that catalyzes the phosphorylation of eEF-2, a 100-kDa protein involved in the translocation step in peptide-chain elongation. Phosphorylation of eEF-2 on Thr56 decreases its affinity for the ribosome, thereby terminating elongation. As shown in Fig. 2, this kinase is exquisitely controlled by mTOR (Browne and Proud, 2004) and AMP-activated protein kinase (AMPK) (Horman et al., 2002; Browne et al., 2004). When energy is abundant, eEF2K is inhibited by the activity of mTOR and protein elongation occurs; in contrast, when energy is deficient, the activity of mTOR is decreased, and eEF2K is activated, thereby inhibiting peptide elongation and conserving

energy. Several lines of evidence suggest that there is a direct link between the activity of eEF2k and autophagy. For example, we found that eEFK can act as a positive regulator of autophagy under environmental or metabolic stresses, including nutrient deprivation (Wu et al., 2006), growth factor inhibition (Cheng et al., 2010), endoplasmic reticulum stress (Cheng et al., 2013), and energy stress caused by the glycolytic inhibitor 2-deoxy-D-glucose (Wu et al., 2009).

b. AMP-activated protein kinase. Activated protein kinase (AMPK), a serine/threonine protein kinase, is a chief sensor of cellular energy and regulates the metabolism of glucose and lipids in response to alterations in nutrient and intracellular ATP concentrations. AMPK is activated when the intracellular AMP/ATP ratio increases. AMPK can be phosphorylated on different sites by various kinases. Phosphorylation of AMPK on Thr172 within its catalytic α subunit is necessary for the activation of this kinase. Recent studies have identified liver kinase B1 (LKB1), the major upstream activating kinase of AMPK, Ca^{2+}/calmodulin-dependent protein kinase kinase-β (CaMKKβ), and transforming growth factor-β-activated kinase (TAK1) as major kinases that phosphorylate AMPK on Thr172 and cause activation of this kinase (Momcilovic et al., 2006; Carling et al., 2008). AMPK also contains other phosphorylation sites in the α-subunit, including Thr258, Ser485, and Ser491, but these phosphorylation sites are not involved in activation of this kinase (Woods et al., 2003). The native β_1-subunit of AMPK is phosphorylated at three sites: Ser24/Ser25, Ser108, and Ser182 (Mitchelhill et al., 1997). It is known that mutation of the Ser108 site leads to the inhibition of AMPK, whereas the mutations on Ser24/Ser25 and Ser182 sites have no effect on the enzyme activity (Warden et al., 2001).

AMPK also participates in the regulation of other cellular functions, including maintenance of mitochondrial homeostasis, cell polarity, and cell growth and proliferation (Hardie, 2011). Studies also have revealed that AMPK is an important regulator of autophagy (Meley et al., 2006; Liang et al., 2007; Matsui et al., 2007; Herrero-Martin et al., 2009). AMPK-mediated suppression of mTOR is controlled by TSC2 and Raptor, two substrates of AMPK. Activation of AMPK by energy deficiency directly phosphorylates TSC2 on T1227 and S1345 and enhances the functions of TSC2, leading to suppression of mTORC1, the key downstream target of TSC1/TSC2 (Inoki et al., 2003). Alternatively, AMPK can regulate the mTOR signaling by directly phosphorylating the mTOR binding partner Raptor on two conserved serine residues, Ser722 and Ser792; these phosphorylations enhance 14-3-3 binding to Raptor, resulting in the inactivation of Raptor and mTORC1 (Gwinn et al., 2008).

AMPK also has a more direct link with autophagy through its interaction with and phosphorylation of ULK1 (Fig. 2). AMPK binds to the N-terminal proline/serine (PS) rich domain of ULK1, and this interaction is required for the ULK1-mediated autophagy (Herrero-Martin et al., 2009). Furthermore, AMPK can associate with and directly phosphorylate ULK1 on several sites, and this modification is required for ULK1 activation in response to nutrient deprivation (Egan et al., 2011). Mack et al. (2012) reported that AMPK can interact with and phosphorylate ULK1 at Ser555, Ser637, and Thr659, and that AMPK-dependent phosphorylation of ULK1 is important for ULK1 regulation of ATG9 localization, resulting in increased autophagy efficiency. Similarly, under glucose starvation, AMPK promotes autophagy by directly phosphorylating ULK1 on Ser317 and Ser777, thus activating ULK1 (Kim et al., 2011a). By contrast, Shang and co-workers found that ULK1 undergoes dramatic dephosphorylation on Ser638 and Ser758 upon starvation, and the dephosphorylation of ULK1 leads to its dissociation from AMPK and becoming more active in autophagy induction (Shang et al., 2011; Shang and Wang, 2011).

c. Bcl-2 family proteins. Bcl-2 family proteins were initially identified and characterized as regulators of cell survival and death; more recently, it has been revealed that the proteins of this family control the autophagic process as well. The antiapoptotic Bcl-2 family members, including Bcl-2, Bcl-XL, Bcl-wl, and Mcl-1, also inhibit autophagy (Fig. 2). Conversely, proapoptotic Bcl-2 homology 3 (BH3)-only proteins such as BNIP3L, Bad, Noxa, Puma, BimEL22, and Bik stimulate autophagy (Maiuri et al., 2009b). For example, the antiapoptotic proteins can interact with Beclin1 through the BH3 receptor domain and the BH3 domain on Belcin1, thereby inhibiting Beclin1-dependent autophagy (Pattingre et al., 2005). The Bcl-2-Beclin1 interaction is inhibited by nutrient deprivation, leading to release of Beclin1 and activation of autophagy. Other proteins with BH3 domains can competitively disrupt the association between Beclin-1 and Bcl-2/Bcl-XL and induce autophagy. Mitophagy induced by hypoxia occurs through a hypoxia inducible factor-1 (HIF-1)–dependent transcriptional activation of BNIP3L that disrupts the interaction between Bcl-2 and Beclin1 (Bellot et al., 2009). It is now widely appreciated that Bcl-2 is not only an antiapoptotic but also an antiautophagic protein.

d. The tumor suppressor p53 protein. p53 is a transcription factor that transactivates proapoptotic and cell cycle–arresting genes and is frequently inactivated or mutated in cancer cells (Vousden and Lane, 2007). It has been found that inactivation of p53 can trigger autophagy in vitro and in vivo (Tasdemir et al., 2008). Tasdemir et al. (2008) found that introduction of wild-type p53 into $p53^{-/-}$ cells inhibited autophagy, whereas forced expression of a nucleus-restricted form of p53 failed to suppress autophagy in the same cells, suggesting that cytoplasmic p53 is responsible for the inhibition of autophagy. In addition, many different

inducers of autophagy stimulate proteasome-mediated degradation of p53, and inhibition of p53 degradation can prevent the activation of autophagy (Tasdemir et al., 2008).

By contrast, nuclear p53 stimulates autophagy in a transcription-dependent fashion through activating the expression of the autophagy-inducing genes such as damage-regulated autophagy modulator (DRAM) (Crighton et al., 2006) and sestrin (Maiuri et al., 2009a). p53 also activates autophagy by inhibiting mTOR in an AMPK-dependent and TSC1/TSC2-dependent manner (Chen and Debnath, 2010). In addition, sestrin 1 and sestrin 2, two p53 target genes, have been identified as a critical link between p53 activation and mTOR inhibition (Budanov and Karin, 2008). Thus, p53 can modulate autophagy in dual ways, depending on its cellular localization; nuclear p53 activates autophagy through transcriptional regulation, whereas cytoplasmic p53 inhibits autophagy (Fig. 2).

III. Relevance of Autophagy to Human Diseases

Depending on the context, autophagy can play either a cytoprotective or cytopathic role in different pathophysiologic processes. Not surprisingly, dysregulation of autophagy can contribute to the pathogenesis of various human diseases, including cancer, obesity, cardiac disease, neurodegeneration, aging, and infectious and inflammatory diseases (Shintani and Klionsky, 2004; Levine and Yuan, 2005; Levine and Kroemer, 2008; Rubinsztein et al., 2012; Liu et al., 2013) (Table 1).

A. Cancer

The association between autophagy and cancer is complex, as the role of autophagy may differ in different stages of disease. At early stages of tumor development, autophagy may prevent genomic instability and suppress growth of precancerous cells, acting as a tumor suppressor (Gozuacik and Kimchi, 2004). Later in the course of disease, autophagy may favor the ability of cancer cells to survive metabolic and therapeutic stresses and thereby promote tumor progression.

1. Autophagy Suppresses Tumor Initiation. It is generally accepted that autophagy has a tumor suppressor function and that defects in autophagy contribute to tumor development. Malignant or transformed cells often display lower basal autophagic activity than their normal counterparts. For example, autophagic activity was lower in primary hepatocellular tumor cells than in normal hepatocytes in rat liver carcinogenesis models (Kisen et al., 1993).

The identification of the genes required for autophagy provides the opportunity to use genetic approaches to investigate the role of autophagy in cancer development. Accordingly, the suppressive effect of autophagy on tumorigenesis has been evaluated using different models that are deficient for specific autophagy factors (Table 1). Mice with a systemic mosaic deletion of *Atg5* or mice with a liver-specific *Atg7* deficiency developed benign tumors in the liver, suggesting that autophagy is critical for suppression of spontaneous tumorigenesis in this model (Takamura et al., 2011). Mice with heterozygous disruption of *Beclin1* showed an increased frequency of spontaneous cancers (Qu et al., 2003; Yue et al., 2003). Beclin1 protein is expressed at a high level in normal breast epithelial cells but has much lower expression in breast cancer cell lines such as MCF-7 (Liang et al., 1999). Ectopic expression of Beclin1 in MCF-7 cells activates autophagy, inhibits cellular proliferation and clonogenicity, and suppresses tumorigenesis in mouse xenograft models (Liang et al., 1999). A functional Beclin1 also inhibits proliferation of other tumor cell lines (Levine and Kroemer, 2008). Monoallelic deletion of *Beclin1* was observed in specimens of human breast, ovarian, and prostate cancers (Aita et al., 1999; Shintani and Klionsky, 2004).

Besides Beclin1, several other components of the autophagic machinery were found to possess tumor suppressor functions. For example, mice deficient in Atg4C showed an increased susceptibility to chemically induced fibrosarcomas (Marino et al., 2007). UVRAG, a positive regulator of the Beclin1-class III PI3K complex, is monoallelically deleted at a high frequency in human colon cancers, and it suppresses the proliferation and tumorigenicity of human colon cancer cells (Liang et al., 2006). Knockout of Bif-1, an inducer of autophagy that interacts with Beclin1 and activates the Beclin1-class III PI3K complex, enhances the development of spontaneous tumors in mice (Takahashi et al., 2007). Thus, autophagy genes may serve, under certain circumstances, as tumor suppressors.

Not only do mutations of the autophagy gene promote tumorigenesis, but autophagy is also positively regulated by the tumor suppressor genes and negatively regulated by the oncogenic signaling pathways. The class I PI3K/Akt signaling pathway promotes cell growth; mutations in several proteins of this pathway are found in high incidence in the common human malignancies. When the Akt signaling pathway is activated, autophagy is often reduced. For instance, Wang et al. (2012) found that Beclin1, an essential autophagy-regulatory protein, is a target of Akt, and that the Akt-mediated phosphorylation of Beclin1 leads to suppression of autophagy and is associated with oncogenesis, providing evidence for the role of the Akt-mediated down-regulation of autophagy in the oncogenic process. Conversely, the tumor suppressor PTEN, which antagonizes the signaling of the PI3K/Akt pathway, can activate autophagy (Arico et al., 2001). Mutations in PTEN result in constitutive activation of the Akt signaling pathway, suppression of autophagy, and tumor formation. Other tumor suppressors such as TSC1, TSC2, p53, and LKB1 stimulate autophagy through their inhibitory effects on

TABLE 1
The phenotypes and diseases associated with alteration of the autophagy genes

Genotype of the Autophagy-Related Genes	Phenotype	Relevant Diseases	Reference
	Protective Autophagy		
Beclin1 deletion	Mice show an increased frequency of spontaneous tumors.	Breast, ovarian, and prostate cancer	Liang et al., 1999; Qu et al., 2003; Yue et al., 2003
Beclin1 overexpression	Decreases occur in MCF-7 cellular proliferation, in vitro clonogenicity, and tumorigenesis in nude mice.	Breast cancer	Liang et al., 1999
Atg5 deletion or the liver-specific Atg7 deficiency	Mice develop benign tumors in the liver.	Liver cancer	Takamura et al., 2011
Atg4C deficiency	Mice show increased chemically induced fibrosarcomas.	Fibrosarcomas	Marino et al., 2007
Bif-1deficiency	Mice show an increased frequency of spontaneous lymphomas.	Lymphomas	Takahashi et al., 2007
UVRAG overexpression	Significant tumor mass reduction occurs in mice.	Colon cancer	Liang et al., 2006
	Protective Autophagy		
Atg7 mutation	Mice show impaired glucose tolerance and a decreased level of serum insulin.	Diabetes	Jung et al., 2008
	Protective Autophagy		
Atg5 deficiency	Mice show cardiac hypertrophy and contractile dysfunction.	Cardiomyopathy	Nakai et al., 2007
LAMP2 deficiency	Cardiac myocytes are ultrastructurally abnormal, and heart contractility is severely reduced.	Danon disease	Nishino et al., 2000; Tanaka et al., 2000
Beclin1 deficiency	Mice show greater intracellular aggregate accumulation and hastened heart failure.	Heart disease	Tannous et al., 2008
Beclin1 or Atg5 overexpression	Enhanced autophagy significantly reduced apoptosis in cardiac HL-1 cells after ischemia/reperfusion.	Heart disease	Hamacher-Brady et al., 2006, 2007
	Autophagic Cell Death		
Beclin1 knockdown	Inhibition of autophagy enhanced cell survival in cardiac myocytes in response to ischemia/reperfusion.	Heart disease	Valentim et al., 2006
Disruption or overexpression of Beclin1	Inhibition or promotion of autophagy diminished or accentuated the pathogenesis of load-induced heart failure in mice.	Cardiac hypertrophy	Zhu et al., 2007
	Protective Autophagy		
Atg7 or Atg5 deficiency	Mice displayed ubiquitinated protein aggregates and inclusion bodies in their neurons.	Neurodegenerative disease	Hara et al., 2006; Komatsu et al., 2006
Ambra1 deficiency	Mice showed severe neural tube defects.	Neurodegenerative disease	Fimia et al., 2007
	Protective Autophagy		
Beclin1 deficiency	Accelerated amyloid-β accumulation occurs.	Alzheimer disease	Pickford et al., 2008
	Protective Autophagy		
Beclin1 overexpression	The accumulation of α-syn and the associated neuronal pathology are reduced in neuronal cells and a mouse model.	Parkinson disease	Spencer et al., 2009
	Protective Autophagy		
Bec-1, Ce-atg7, or Ce-atg18 knockdown	The aggregate formation and toxicity of polyQ expansion proteins were increased in *C. elegans*.	Huntington disease	Jia et al., 2007
	Protective Autophagy		
Beclin 1 overexpression	The brains of mice infected with Sindbis virus have fewer Sindbis virus RNA-positive cells, fewer apoptotic cells, and lower viral titers	Infectious disease	Liang et al., 1998
Atg5 deficiency	Dendritic cells show impaired autophagic delivery to endosomal Toll-like receptors and interferon production during virus infection.	Infectious diseases	Lee et al., 2007
Atg5 deficiency	Periphery $CD8^+$ T cells display increased apoptosis, and $CD4^+$ and $CD8^+$ T cells show defective activation-induced proliferation.	Infectious diseases	Pua et al., 2007
	Protective Autophagy		
Bec-1 or Ce-atg7 deficiency	The mean and maximum life span of *C. elegans* were decreased.	Aging	Jia and Levine, 2007
	Protective Autophagy		
IRGM and ATG16L1	A genomewide association scan in individuals with Crohn disease identified IRGM and ATG16L1 as new risk loci.	Immune disease	Massey and Parkes, 2007; Parkes et al., 2007
	Protective Autophagy		
Beclin1 overexpression	Restoration of autophagy rescued the disease phenotype in cystic fibrosis epithelial cells.	Inflammation	Luciani et al., 2010

mTOR (Rubinsztein et al., 2012). Therefore, not only do autophagy genes control the autophagic activity, but oncogenes and tumor suppressor genes can also modulate this cellular process through which tumor initiation and development are influenced.

The mechanisms by which autophagy suppresses tumor initiation and development appear to include limiting the accumulation of damaged proteins and organelles such as mitochondria; preventing genomic instability and subsequent cellular transformation (Jin, 2006; Mathew et al., 2007); and limiting the inflammation caused by necrosis (Degenhardt et al., 2006). As in the case of chronic infection and toxin exposure, defects in the autophagic disposal can cause chronic tissue damage and inflammation, which may contribute to cancer (White et al., 2010; Amaravadi et al., 2011). Also, autophagy can inhibit tumor growth by degrading some proteins required for cell proliferation. Alternatively, autophagy may protect against tumor initiation and development by favoring cellular differentiation, increasing protein catabolism, or promoting autophagic cell death.

2. Autophagy Promotes Tumor Progression. As tumors enlarge, cancer cells may face metabolic stresses such as nutrient and growth factor deprivation or hypoxia due to insufficient vascularization. In addition, cancer treatments such as radiation, chemotherapy, and so-called target therapies, including those that disrupt angiogenesis inhibit the function of proteasomes or disrupt signaling pathways, can create other forms of cellular stress. Under these circumstances, autophagy may protect the viability of tumor cells by providing nutrients and energy through degradation of cytoplasmic components for the recycling of amino acids and fatty acids. It has been demonstrated that the blunting of autophagy by impeding the early stage of autophagy using 3MA, the inhibiting the formation of autophagolysosomes using hydroxychloroquine (HCQ) or bafilomycin A1, or the silencing of Atg5, Beclin1, Atg10, or Atg12 expression by RNA interference can all facilitate the death of starved cancer cells (Boya et al., 2005). Autophagy may protect cancer cells against the oxidative stress and DNA damage caused by chemotherapeutic agents by eliminating damaged macromolecules or organelles, allowing the continued survival of cancer cells and thereby contributing to drug resistance. Inhibition of autophagy has been shown to enhance the efficacy of anticancer drugs (Janku et al., 2011).

Some types of cancer cells, particularly those harboring an activated Ras oncogene, depend on autophagic activity for survival even in the absence of external stressors (Mancias and Kimmelman, 2011). Both in vitro and in vivo studies have found that expression of oncogenic Ras increased autophagy, which may support transformation and tumor growth through adapting to cellular stresses and maintaining energy levels (Guo et al., 2011; Kim et al., 2011b; Lock et al., 2011; Yang et al., 2011a). Moreover, after radiotherapy or chemotherapy, constitutive up-regulation of autophagy may favor a dormancy state in residual cancer cells, including cancer stem cells; this may contribute to tumor recurrence and progression (Lu et al., 2008; White et al., 2010). Senescence, a state of cell-cycle arrest that may serve as a tumor suppressor, has been suggested as a mechanism underlying tumor dormancy mediated by autophagy (Gewirtz, 2009). It was demonstrated that autophagy mediates Ras-induced senescence, and that inhibition of autophagy delays senescence in cancer cells (Young et al., 2009).

3. Future Horizons of Autophagy in Cancer. Autophagy appears to be a double-edged sword in cancer, having both tumor-suppressing and tumor-promoting properties at different stages of cancer. On the one hand, promoting autophagy to prevent persistent tissue damage and chronic inflammation might be beneficial in the setting of cancer prevention; on the other hand, blocking the autophagy-mediated survival of tumor cells is probably advantageous in the treatment of cancer. Thus, modulation of autophagy appears to be a promising but complex new approach to cancer prevention and treatment, although its benefits can be context dependent. Determining how to modulate autophagy as a cancer therapy is an exciting challenge that will provide new insights into cancer biology and approaches to enable cancer eradication.

B. Obesity and Diabetes

Obesity is the phenotypic expression of an imbalance of energy homeostasis. This imbalance is accompanied by the emergence of metabolic and oxidative stress, which may lead to inflammatory responses and organelle dysfunction. Chronic exposure to high energy through nutrient intake increases the demand on synthetic and degradative machinery in tissues such as the liver, adipose tissue, and pancreas, all central of metabolic homeostasis.

Recent studies have shed light on the role of autophagy in the regulation of lipid metabolism and insulin sensitivity. Ineffective handling of macromolecules such as lipids or glycogen could compromise the metabolic function of the liver and blunt the action of insulin (Yang et al., 2010). Yang et al. (2010) found a down-regulation of autophagy in hepatocytes in both genetic and dietary models of obesity. Suppression of *Atg7* expression both in vitro and in vivo results in defective insulin signaling and elevated endoplasmic reticulum stress. By contrast, restoration of *Atg7* expression in the liver limited endoplasmic reticulum stress and enhanced the action of insulin and systemic glucose tolerance in the obese mice (Yang et al., 2010). These observations indicate that autophagy is an important component of insulin signaling and that

reduced autophagy and increased endoplasmic reticulum stress may lead to insulin resistance.

Autophagy has a critical role in normal adipogenesis, and inhibition of autophagy has antiobesity and insulin-sensitizing effects (Goldman et al., 2010). Mice with *Atg7* deletion in the adipose tissues exhibited enhanced insulin sensitivity and higher levels of basal physical activity, and were resistant to obesity while fed a high-fat diet (Zhang et al., 2009).

Obesity increases the risk of type 2 diabetes. In obesity, the size and number of adipocytes increase, resulting in expansion of adipose tissues. Gain of adipose tissues, in particular visceral adiposity, causes adipocyte dysfunction, which may play a role in the development of insulin resistance and other obesity-linked complications (Lavallard et al., 2012). Type 2 diabetes is characterized by glucose intolerance, insulin resistance, and relative insulin deficiency (Meijer and Codogno, 2006; Jung et al., 2008). *Atg7* mutant mice show impaired glucose tolerance, inappropriate concentration of circulating insulin, and reduced β-cell mass and pancreatic insulin content. This evidence indicates that autophagy is necessary for maintenance of the structure, mass, and function of pancreatic β cells. It further indicates that impaired autophagy may contribute to insulin deficiency and hyperglycemia, resulting from abnormal turnover and function of cellular organelles (Ebato et al., 2008; Jung et al., 2008).

C. Cardiovascular Diseases

In the myocardium, autophagy occurs at low levels under normal conditions, and defects in this process will cause cardiac dysfunction and heart failure. Under stressful conditions, autophagy is rapidly increased and plays a protective role to promote cell survival. Excessive autophagy, however, may lead to cell death (Rothermel and Hill, 2008; Gustafsson and Gottlieb, 2009).

Increasing evidence suggests that defective autophagy is associated with heart disorders, including congestive heart failure, cardiomyopathy, and the Danon disease (Table 1). Accumulation of autophagosomes was observed in myocardial biopsy samples from patients with the cardiovascular diseases such as coronary artery disease, hypertension, aortic valvular disease, and congestive heart failure (Terman and Brunk, 2005). It was reported that basal autophagy helps maintain cardiomyocyte function and survival. For example, myocardial-specific deficiency of the autophagy gene *Atg5* causes cardiomyopathy in adult mice characterized by hypertrophy and contractile dysfunction (Nakai et al., 2007). In cultured cardiac myocytes, induction of autophagy reduces the accumulation of misfolded proteins and other intracellular aggregates that are known to contribute to cardiac diseases (Pattison and Robbins, 2011). Mice with deficiency of LAMP2 show excessive accumulation of autophagic vacuoles and impaired autophagic degradation of long-lived proteins, which may contribute to the incidence of cardiomyopathy (Nishino et al., 2000; Tanaka et al., 2000).

Apart from its apparent role in maintaining the normal functions of the heart, autophagy is also an adaptive response that protects the heart from various stresses. Activation of autophagy in the stressed heart provides energy substrates and promotes cellular remodeling. It is believed that autophagic activity in cardiac myocytes provides a necessary energy source during the period between birth and suckling (Kuma et al., 2004). It has been found that autophagy triggered by ischemia is a homeostatic mechanism that reduces apoptosis and the deleterious effects of chronic ischemia (Yan et al., 2005). Inhibition of autophagy hastens heart failure, accelerates ventricular dysfunction, and increases mortality in mice harboring the αB-crystalin mutant, indicating that autophagy acts as an adaptive response in this proteotoxic model of heart disease (Tannous et al., 2008).

Autophagy is a protective response to toxic lipopolysaccharides in neonatal rat cardiomyocytes through which damaged organelles are removed (Hickson-Bick et al., 2008). It has been found that the removal of depolarized mitochondria by mitophagy plays a critical role in protecting the cardiac muscle cells subjected to ischemic stress (Huang et al., 2011). Cardiac-specific deficiency of the *Atg5* gene does not cause any phenotypic abnormalities during the early stages of cardiogenesis under normal conditions, but results in severe cardiac dysfunction after treatment with pressure overload or β-adrenergic stress (Nakai et al., 2007). Recently, it has been reported that autophagy plays a protective role in HL-1 myocardial cells subjected to ischemia/reperfusion (I/R), and that inhibition of autophagy renders these cells more sensitive to apoptosis after I/R (Hamacher-Brady et al., 2006). Consistent with this, it was observed that up-regulation of autophagy induced by I/R constitutes a protective response against BNIP3-mediated apoptotic cell death through removal of damaged mitochondria (Hamacher-Brady et al., 2007).

There is also the apparently contradictory report that excessive induction of autophagy underlies autophagic cell death and loss of cardiomyocytes (Martinet et al., 2007a) (Table 1). This hypothesis is supported by reports that dead and dying cardiomyocytes showed the characteristics of autophagy, and that increased autophagy was associated with heart failure (Takemura et al., 2006). Akazawa et al. (2004) provided evidence suggesting that the loss of myocardial cells caused by autophagic cell death plays a causal role in the pathogenesis of heart failure. They observed that degenerated cardiomyocytes of the mouse heart exhibited characteristics indicative of autophagic cell death. Several studies have reported that induction of autophagy contributes to cell death during I/R. Valentim et al. (2006) found that inhibition of autophagy by

knockdown of Beclin1 or treatment with 3-MA enhanced cell survival in the cardiac myocytes treated with I/R. Activation of autophagy in cardiomyocytes may also contribute to heart failure caused by pressure overload (Zhu et al., 2007), as evidenced by the observation that inhibition of autophagy can diminish pathologic remodeling induced by severe pressure stress. Conversely, increased autophagic activity promotes pathologic remodeling (Zhu et al., 2007).

In summary, cardiac autophagy appears to function predominantly as a prosurvival mechanism under normal or mildly stressed conditions. When excessive autophagy is present, however, the autophagic machinery may play a self-destructive role, leading to damaged myocardium and congestive heart failure.

D. Neurodegenerative Diseases

The role of autophagy in several neurodegenerative diseases, including Alzheimer disease (AD), Parkinson disease (PD), and Huntington disease (HD) (Rubinsztein et al., 2012), has received recent attention. Despite the apparently distinct etiologies of these neurodegenerative disorders, pathologically they share the presence of protein inclusions and aggregates within neurons (Rubinsztein, 2006). Thus, failure of protein degradation may be one cause of the neuronal cell death in these neurodegenerative disorders.

Despite the morphologic evidence of autophagy activity in the neurons from patients with AD, PD, and HD (Levine and Yuan, 2005; Mizushima, 2007), the role of autophagy in neurodegenerative disorders remains unclear. As in other circumstances we have described, it is not always easy to decipher whether the presence of autophagy is a survival response or a form of programmed cell death. Autophagy is seen in neurons undergoing neurodegeneration, but is this a death response or a failed attempt at survival? These observations suggest a role for autophagy in neurodegeneration. Interventions that block autophagy and increase cell death would suggest a survival role, whereas those that decrease cell death would suggest the opposite. Thus, further experimental and clinical studies that ascertain where autophagy plays a role should be of high priority.

In contrast, there have been studies reporting that accumulation of autophagosomes (indicative of activation of autophagy) acts as a protective mechanism to degrade mutant or toxic proteins. Experiments have shown that reduced autophagic activity could result in disturbance of the degradation of intracellular protein and the formation of the protein aggregates and inclusions that may cause cellular toxicity and disrupt neural function (Hara et al., 2006; Komatsu et al., 2006). Induction of autophagy can enhance the clearance of cytoplasmic aggregate-prone proteins. Accumulation of ubiquitin aggregates was detected in the central nervous system of mice lacking *Atg7* and was accompanied by neurodegeneration, abnormal neurologic signs, and death (Komatsu et al., 2006). Similarly, mice deficient for *Atg5* develop progressive deficits in motor function accompanied by the accumulation of cytoplasmic inclusion bodies in neurons (Hara et al., 2006). Ambra1, a component of the Beclin1-class III PI3K complex, is a positive regulator of autophagy, and its deficiency in mouse embryos leads to severe neural tube defects associated with uncontrolled cell proliferation (Fimia et al., 2007).

Following the morphologic evidence of autophagy in neurodegenerative disorders like AD, PD, and HD (Mizushima, 2007), more studies demonstrated the roles of autophagy in these pathologic conditions. PD, the second most common neurodegenerative disorder, is an age-related disease characterized by resting tremor, slowed movement, postural instability, and muscle rigidity (Janda et al., 2012). Autophagy has been found to play an important role in the intracellular clearance of α-synuclein (α-syn), a major component of the neuronal cytoplasmic inclusions (Lewy bodies) that characterize PD (Webb et al., 2003). Overexpression of Beclin1 reduced the accumulation of α-syn and ameliorated the related neuronal pathologic features in a mouse model (Spencer et al., 2009). Recent studies have linked mitophagy defects to PD, which is associated with the accumulation of mitochondrial damage. For example, PINK1 and parkin have critical roles in the mitophagic removal of the damaged mitochondria, and mutations in these genes are linked with autosomal recessive PD (Cesari et al., 2003; Vives-Bauza et al., 2010).

AD is the leading cause of dementia in the aging population, and a close link has been shown between defects of autophagy and AD. It has been observed that expression of Beclin1 was significantly reduced in the brains of patients with AD, and that Beclin1 deficiency not only reduced autophagic activity in neurons but also accelerated β-amyloid accumulation and promoted neurodegeneration. By contrast, up-regulation of Beclin1 decreased amyloid deposition. Thus, restoring autophagy may represent an effective approach to treatment of AD (Pickford et al., 2008).

HD is a neurodegenerative genetic disorder caused by the expansion of the polyglutamine (polyQ) tract in huntingtin (Htt). This results in the death of striatal medium spiny neurons, with subsequent disruption of both basal ganglia and higher function, eventually leading to the characteristic choreiform movements, cognitive decline, and psychiatric problems. Autophagy is implicated in regulating the turnover of Htt (Sarkar et al., 2007b), the main component of the nuclear and cytosolic inclusions detected in the HD-affected neurons. Knockdown or knockout of the *Atg* genes increases the formation of aggregates and toxicity of the polyQ expansion proteins in *Caenorhabditis elegans* (Jia et al., 2007).

Taken together, autophagy plays a protective role against the neurodegenerative diseases, and defects in autophagy are an important contributor to these disorders. Thus, it can be expected that modulating autophagy activity by a pharmacologic approach or other means might emerge as a novel therapeutic strategy for the neurodegenerative diseases.

E. Aging

The process of aging is closely associated with accumulation of damaged proteins and organelles, in particular, the mitochondria. It has been shown in *C. elegans* and rodents that decreased generation of damaged proteins and organelles is associated with extension of the life span (Levine and Klionsky, 2004). Accumulation of damaged proteins and mitochondria may reflect an insufficiency of protein turnover, which may then contribute to the aging process. Autophagy participates in eliminating damaged proteins and organelles, reducing reactive oxygen species (ROS) production, and promoting mitochondrial turnover and biogenesis; all of these may contribute to life-span extension and prevent or delay the development of the complications associated with aging (Rubinsztein et al., 2011). Normal and pathologic aging are often associated with a reduced autophagic degradation, which may result in an accumulation of protein aggregates and damaged mitochondria, thus contributing to age-related cellular dysfunctions (Cuervo, 2008).

In fact, the role of autophagy in increasing life spans has been studied in various model systems such as yeast (Matecic et al., 2010), *C. elegans* (Toth et al., 2008), and fruit flies (Simonsen et al., 2008). Activation of autophagy has potent antiaging effects, and inhibition of this cellular process accelerates aging and compromises the longevity-promoting effects of caloric restriction (CR) (Rubinsztein et al., 2011). CR is a key antiaging intervention shown to contribute to extension of life span in diverse eukaryotic organisms including yeast, flies, worms, rodents, and mammals (Colman et al., 2009), and to be beneficial to human health. CR is a potent inducer of autophagy, and inhibition of autophagy prevents the antiaging effects of CR (Rubinsztein et al., 2011), suggesting that the effects of CR on longevity are mediated at least in part through activating autophagy. Jia and Levine (2007) reported that the autophagy genes *bec-1* and *Ce-atg7* are required for the longevity effects of dietary restriction in *C. elegans*, providing the first genetic evidence for the role of autophagy in the extension of the life span. Inhibition of mTOR can also extend the life span in several model organisms and confer protection against a variety of age-related pathogeneses; multiple mTORC1-regulated processes, such as mRNA translation and autophagy, likely contribute to the prolongevity effects of mTORC1 inhibition (Johnson et al., 2013). Evidence from studies in yeast and invertebrates shows that the mTORC1-regulated autophagy is required for the life-span extension caused by dietary restriction or by rapamycin administration (Alvers et al., 2009; Bjedov et al., 2010). It is believed that there exists a cross-link between the molecular mechanisms regulating autophagy and aging. CR induces autophagy through the activation of Sirt1 and/or the inhibition of mTOR. Signaling pathways involved in stress-resistance and longevity, such as FoxO3 and p53, are also involved in autophagy regulation (Salminen and Kaarniranta, 2009).

F. Infectious Diseases

Induction of autophagy may also be beneficial in the treatment of infectious diseases, as this cellular process can defend against microorganisms via selective degradation of pathogens and activation of innate and adaptive immunity (Levine et al., 2011). It was observed that autophagic activity was increased during host infection by various bacteria and viruses (Rikihisa, 1984; Munz, 2009). In the *C. elegans* and *Dictyostelium discoideum* models, mutations in autophagy genes that diminish autophagy render the hosts more susceptible to infection by invasive bacteria and permit intracellular bacterial replication (Jia et al., 2009). Overexpression of Beclin1 can inhibit replication of the Sindbis virus and protect cells against infection, suggesting that autophagy plays a role in host defense against this viral infection (Liang et al., 1998).

The autophagic machinery is used in the selective delivery of microorganisms to lysosomes in a process called xenophagy (Mizushima et al., 2008). This cellular process can degrade various intracellular and invading pathogens such as bacteria and viruses (Kirkegaard et al., 2004; Nakagawa et al., 2004). In addition to degrading infectious agents, activation of autophagy enhances antimicrobial innate and adaptive immune signaling (Deretic, 2005). Autophagy may function as an innate effector against mycobacterial infection through the receptor-dependent or/and receptor-independent pattern-recognition mechanisms (Fabri et al., 2011). By degrading intracellular pathogens and cytotoxic microbial virulence products, autophagy may support cellular survival during pathogen invasion.

However, induction of autophagy may also aid bacteria and viruses to invade host tissues and escape host defense mechanisms. Bacteria such as *Porphyromonas gingivalis* and *Brucella abortus* enter cells and survive within autophagosomal vacuoles. During infection, these bacteria are internalized into the early endosome-like vacuoles and then sequestered into the autophagosomes, thereby inhibiting fusion of autophagosome with lysosome and preventing the degradation by lysosomal hydrolases (Dorn et al., 2002; Marino and Lopez-Otin, 2004). Autophagy may also provide pathogens with nutrients for growth, and with substrates for metabolic pathways. Additionally, autophagy is used by

certain types of viruses during replication. For example, Prentice et al. (2004) reported that cellular autophagy was required for replication of the corona virus and mouse hepatitis virus (MHV). Therefore, autophagy can either be favorable or unfavorable to pathogen infection.

Our current knowledge of the role of autophagy in infection suggests that, depending on the type of pathogen, the autophagic machinery can either be used by viruses or bacteria to infect and to grow within host cells, or the autophagic process can function as a protective mechanism against infection. Therefore, as in many other circumstances, autophagy may have both favorable and unfavorable effects. Exploiting autophagy to devise new strategies for treatment of infectious diseases appears to be promising but also challenging, and will largely depend on a more complete understanding of the role of autophagy in different types and stages of infection.

G. Immune Diseases

In addition to degrading pathogens as previously discussed, up-regulation of autophagy may facilitate optimal regulation of innate and adaptive immune signaling (Levine and Deretic, 2007; Kuballa et al., 2012). Autophagy participates in trafficking events that deliver microbial nucleic acids and antigens to endo/lysosomal compartments for activation of innate immunity (Schmid and Munz, 2007). The delivery of viral pathogens to Toll-like receptor 7 (TLR7) was impaired and the secretion of both interferon α (IFN-α) and interleukin-12 (IL-12) were decreased in the *Atg5*$^{-/-}$ dendritic cell model (Lee et al., 2007). An efficient immune response against pathogens involves complex intracellular antigen-processing events.

It has been found that autophagy may benefit the host by degrading endogenous viral proteins for antigen presentation. For example, during herpes simplex virus 1 (HSV-1) infection, autophagy increases the presentation of endogenous viral antigens to CD8$^+$ T cells on the major histocompatibility complex (MHC) class I molecule (English et al., 2009). Autophagy also may enhance presentation of major histocompatibility complex class II to CD4$^+$ T cells (Schmid et al., 2007) and is an important mechanism controlling the homeostasis of CD4$^+$ T cells (Li et al., 2006). Autophagy appears to be involved in the development and survival of T lymphocytes. For example, *Atg5*$^{-/-}$ CD8$^+$ T lymphocytes display dramatically increased cell death, and *Atg5*$^{-/-}$ CD4$^+$ and CD8$^+$ T cells fail to undergo efficient proliferation after T-cell receptor stimulation (Pua et al., 2007).

Not only does the autophagic machinery function in innate and adaptive immunity, but several surface receptors and cytokines involved in innate and adaptive immune responses also stimulate autophagy for the clearance of intracellular pathogens (Levine and Kroemer, 2008). For instance, Toll-like receptors activate autophagy in response to pathogen invasion (Delgado et al., 2008). Cytokines of both of innate and adaptive immunity have been reported to regulate autophagy, including IFN-γ, TNF-α, IL-4, and IL-13. Among these, IFN-γ (Inbal et al., 2002) and TNF-α (Djavaheri-Mergny et al., 2006) stimulate autophagy, whereas IL-4 and IL-13 block autophagy (Harris et al., 2007).

Autophagy is also linked to other immune functions such as the secretion of proinflammatory cytokines. Autophagy can mitigate inflammatory reactions through different mechanisms. The rapid removal of apoptotic corpses by autophagy ameliorates tissue inflammation (Levine and Kroemer, 2008). Autophagy-deficient *Atg5*$^{-/-}$ embryos demonstrate increased inflammation in tissues where there is an impaired clearance of apoptotic cells (Qu et al., 2007). *Atg16L1*-deficient macrophages overproduce the inflammatory cytokines IL-1β and IL-18 after treatment with lipopolysaccharides, suggesting that autophagy is involved in the control of endotoxin-induced inflammatory immune responses (Saitoh et al., 2008). Autophagy can also inhibit the activation of the NLRP3 "inflammasome" and the secretion of IL-1β and IL-18, likely through improving the quality control of the mitochondria and removing ROS and the cytosolic mitochondrial DNA (Nakahira et al., 2011). Consistently, it was found that inhibition of mitophagy leads to the accumulation of damaged, ROS-generating mitochondria, and that this is responsible for activating the NLRP3 inflammasome (Zhou et al., 2011).

Our knowledge of the role of autophagy in immunity has been further strengthened by connections found between the Crohn disease and ATG16L and autophagy-stimulatory immunity-related GTPase (IRGM), two genes involved in autophagy regulation (Massey and Parkes, 2007; Parkes et al., 2007). These studies suggest a potential role for the deregulation of autophagy in the pathogenesis of Crohn disease. Genomewide studies have linked several single-nucleotide polymorphisms in the *Atg5* gene to susceptibility to systemic lupus erythematosus (SLE), suggesting an involvement of autophagy in this autoimmune disease (Levine et al., 2011). Another connection between autophagy and chronic inflammation was found in cystic fibrosis, a life-threatening genetic disorder caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) (Luciani et al., 2010). A defective cystic fibrosis transmembrane conductance regulator results in deficient autophagy in lung epithelial cells; restoration of autophagy by overexpressing Beclin1 can rescue the disease phenotype.

IV. Pharmacologic Modulation of Autophagy: Novel Therapeutic Intervention

A. Mechanism of Action of Autophagy Modulators

Understanding the role of autophagy in healthy tissues has helped identify the importance of autophagy in disease. To develop effective autophagy-based

therapy, it will be essential to identify the key targets in the autophagy pathway for the development of new therapeutic agents. As discussed earlier, autophagy can play a protective or destructive role in disease states, and thus for therapeutic purposes it would be valuable to identify and develop pharmacologic agents that can induce or inhibit this cellular process. A variety of potentially "druggable" targets affecting autophagy have been identified such as eEF2K, which acts as a positive regulator of autophagy under various stresses, as reported by our laboratory (Wu et al., 2009; Cheng et al., 2011a, 2013).

1. Autophagy Inhibitors. Numerous studies have demonstrated that autophagy can be inhibited pharmacologically through targeting the early or late stage of the autophagic process (Fig. 3). Table 2 lists the compounds identified as inhibitors of autophagy. Like all drugs, the potency, activity, selectivity, and safety as well as other pharmacologic properties such as pharmacokinetics, absorption, distribution, metabolism, and elimination are essential elements for a drug's ultimate clinical utility. The inhibitors that target the early stage of autophagy include 3-MA, wortmannin, and LY294002 [2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one hydrochloride], all of which inhibit the class III PI3K (Vps34) and disable the formation of autophagosome. The inhibitors that act on the late stage of autophagy include chloroquine (CQ), bafilomycin A1, leupeptin, and pepstatin. These latter compounds either inhibit the fusion of autophagosomes with lysosomes or the degradation of autolysosomes. The lysosomotropic agents such as CQ and its derivative HCQ can increase lysosomal pH and prevent the digestive activity of hydrolases, leading to inhibition of both the fusion of the autophagosome with the lysosome and the degradative activity of the autolysosomes (Amaravadi et al., 2011). Bafilomycin A1 is a selective inhibitor of the vacuolar H^+ ATPase responsible for acidifying lysosomes.

The digestive phase of autophagy can also be blocked by inhibitors of lysosomal proteolysis such as leupeptin and pepstatin. Autophagosomes and lysosomes move along the microtubules to fuse, so microtubule-disrupting agents, including nocodazole, colchicine, and vinblastine, can block the fusion of autophagosomes with lysosomes (Yang et al., 2011c; Rubinsztein et al., 2012). 2-Phenylethynesulfonamide (PES), a small molecule inhibitor of heat shock protein 70 (HSP70), impairs autophagy

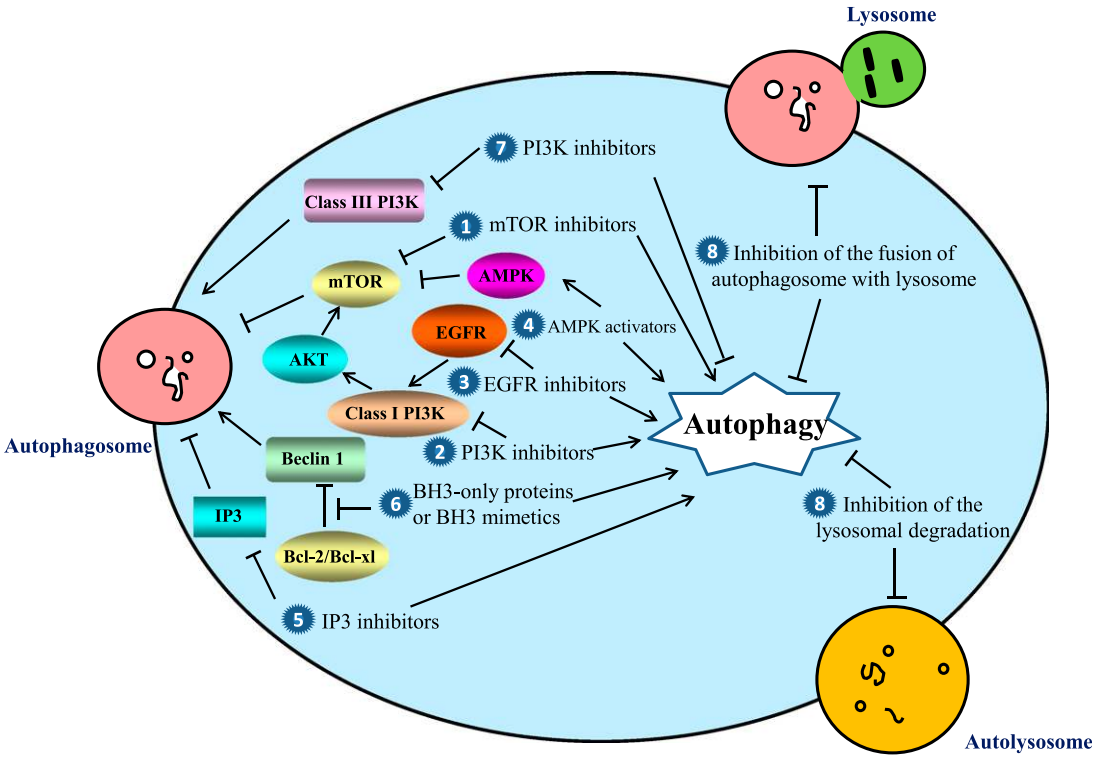
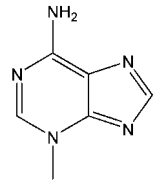
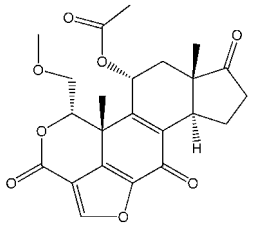
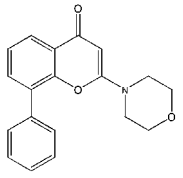
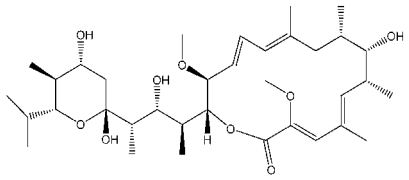
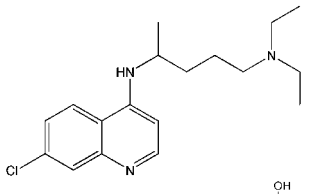
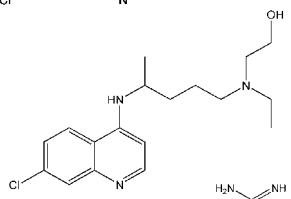
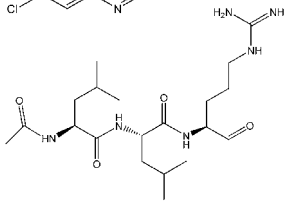
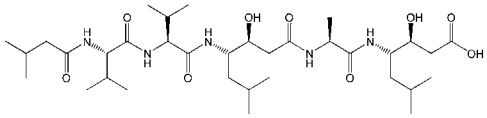
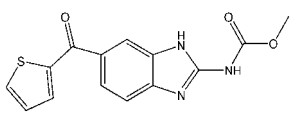


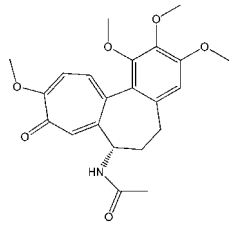
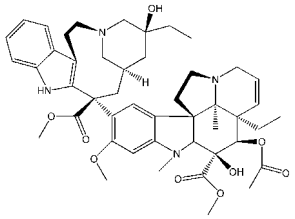
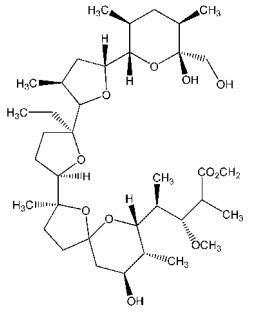
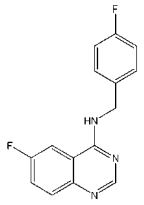
Fig. 3. Autophagy can be targeted at multiple points on its regulatory pathways. (1) Activation of mTOR inhibits induction of autophagy, thus mTOR inhibitors are strong inducers of autophagy. (2) As the activity of mTOR is regulated by the class I PI3K-AKT pathway, the inhibitors of PI3K can stimulate autophagy. (3) EGFR is an upstream regulator of the class I PI3K/Akt pathway, so inhibition of EGFR activates autophagy through suppressing mTOR. (4) AMPK activates autophagy via suppressing mTOR or directly stimulating Ulk1 activity, thus activators of AMPK can induce autophagy. (5) The intracellular level of IP3 negatively regulates autophagy via an mTOR-independent mechanism; inhibiting this pathway can induce autophagy. (6) The antiapoptotic proteins Bcl-2 and Bcl-XL can interact with Beclin 1 through the Bcl-2 homology 3 (BH3) domain, thereby inhibiting Beclin1-dependent autophagy; thus, the small molecule mimetics of BH3 can activate autophagy by blocking the interaction between Bcl-2 and Beclin1. (7) Class III PI3K is critical for autophagy initiation, so its inhibitors can suppress autophagy by blocking the formation of autophagosome. (8) Inhibitors of the lysosomal enzymes and lysosomotropic agents that elevate the lysosomal pH can inhibit autophagy through blocking fusion of the autophagosome with the lysosome and degradation of the autolysosome.

TABLE 2
Compounds known to inhibit autophagy

Type of Agents	Mechanism of Action	Compound	Structure
Inhibitor of class III PI3K	Inhibits the formation of autophagosome	3-Methyladenine	
		Wortmannin	
		LY294002	
Inhibitor of the acidification of lysosome	Increases the pH and inhibits autophagosome-lysosome fusion and the digestive activity of lysosome	Bafilomycin A1	
		Chloroquine	
Inhibitor of the acidification of lysosome	Increases the pH and inhibits autophagosome-lysosome fusion and the digestive activity of lysosome	Hydroxychloroquine	
Inhibitor of lysosomal proteolysis	Prevents the degradation of lysosome	Leupeptin	
		Pestatin A	
Inhibitor of microtubule formation	Blocks the fusion of autophagosome with lysosome	Nocodazole	

(*continued*)

TABLE 2—*Continued*

Type of Agents	Mechanism of Action	Compound	Structure
		Colchicine	
		Vinblastine	
Inhibitor protein transport	Blocks the fusion of the autophagosome and lysosome	Monensin	
Inhibitor of ubiquitin-specific peptidases	Promotes the degradation of Vps34 complexes by enhancing Beclin1 ubiquitination	Spautin-1	

through its inhibitory effects on lysosomal functions (Leu et al., 2009). Monensin, an inhibitor of protein transport, inhibits autophagy by preventing the fusion of the autophagosome with the lysosome (Kondo et al., 2005). Using an imaging-based screen, Liu et al. (2011) recently identified a highly potent small molecule inhibitor of autophagy they named spautin-1 (specific and potent autophagy inhibitor 1), which promotes degradation of the Vps34 complexes via inhibiting ubiquitin-specific processing protease 10 (USP10) and USP13, two ubiquitin-specific peptidases that target the deubiquitination of Beclin1.

2. Autophagy Activators. mTOR, inositol 1,4,5-trisphosphate (IP3), epidermal growth factor receptor (EGFR), Bcl-2, and Bcl-xl negatively regulate autophagy. Thus, drugs targeting these proteins have the capacity to induce autophagy (Fig. 3). Table 3 lists the compounds that have been identified as activators of autophagy. The mTORC1 inhibitor rapamycin and its analogs temsirolimus (CCI-779), everolimus (RAD-001), and deforolimus (AP-23573) are strong inducers of autophagy (Rubinsztein et al., 2012), as are the ATP-competitive inhibitors of mTOR such as Torin1 [1-[4-(4-propanoylpiperazin-1-yl)-3-(trifluoromethyl)phenyl]-9-quinolin-3-ylbenzo[h][1,6]naphthyridin-2-one] (Thoreen et al., 2009) and AZD8055 [[5-[2,4-bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl]-2-methoxyphenyl]methanol] (Chresta et al., 2010), which inhibit both mTORC1 and mTORC2. Some newly developed ATP-competitive inhibitors of mTOR, such as PP242 [2-(4-amino-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1*H*-indol-5-ol], PP30 [3-(4-amino-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-*N*-(4,5-dihydrothiazol-2-yl)benzamide] (Feldman et al., 2009), and WYE-125132 [WYE-132, 1-[4-[1-(1,4-dioxaspiro[4.5]decan-8-yl)-4-(8-oxa-3-azabicyclo[3.2.1]octan-3-yl)pyrazolo[3,4-d]pyrimidin-6-yl]phenyl]-3-methylurea] show stronger

inhibitory activity compared with rapamycin (Yu et al., 2010), but their autophagy-inducing efficacy has not been well documented.

By use of an automated cell-based assay, Balgi et al. (2009) found that rottlerin (a potent large-conductance potassium channel opener and an inhibitor of protein kinase C) and three approved drugs, perhexiline (a prophylactic antianginal agent), niclosamide (a teniacide), and amiodarone (an antiarrhythmic agent), activate autophagy by inhibiting mTORC1 signaling. The activity of mTOR is regulated by the class I PI3K-Akt pathway, and negative feedback between the mTORC1 and the PI3K-Akt pathways exists (O'Reilly et al., 2006). The dual inhibitors of PI3K-mTOR signaling, such as NVP-BEZ235 [2-methyl-2-{4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl]phenyl}propanenitrile] and PI-103 [3-4-(4-morpholinylpyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidin-2-yl-phenol], are potent inducers of autophagy (Fan et al., 2010). As IP3 and IP3R suppress autophagy, inhibition of IP3 by lithium or L-690,330 [[1-(4-hydroxyphenoxy)-1-phosphonoethyl]phosphonic acid] or by xestospongin B (a chemical antagonist of IP3 receptor) stimulates autophagy (Criollo et al., 2007).

EGFR is an upstream regulator of the class I PI3K/Akt pathway known to suppress autophagy through activating mTOR; therefore, induction of autophagy might contribute to the effects of EGFR-targeted therapy. Indeed, cetuximab, a therapeutic antibody that blocks the function of EGFR, induces autophagy in cancer cells (Li and Fan, 2010). The small molecule mimetics of BH3, ABT-737 [*N*-{4-[4-(4′-chloro-biphenyl-2-ylmethyl)-piperazin-1-yl]-benzoyl}-4-(3-dimethylamino-1-phenylsulfanylmethyl-propylamino)-3-nitro-benzenesulfonamide] (Maiuri et al., 2007a) and HA14-1 [2-amino-6-bromo-*a*-cyano-3-(ethoxycarbonyl)-4*H*-1-benzopy ran-4-acetic acid ethyl ester] (Kessel and Reiners, 2007), inhibit the interaction between Bcl-2/Bcl-xl and Beclin1 and increase autophagic activity (Fig. 3 and Table 3).

Autophagy can also be pharmacologically induced via activating the positive regulators of autophagy. Metformin, a widely used antidiabetic agent, activates AMPK and induces autophagy (Yang et al., 2011b). In addition, many other drugs activate autophagy through diverse or even unknown mechanisms of action. For example, some cytotoxic agents have been shown to induce autophagy in cancer cells; these agents include TNF-related apoptosis-inducing ligand (Han et al., 2008), histone deacetylase inhibitors vorinostat (SAHA) (Shao et al., 2004), LAQ824 [(2*E*)-*N*-hydroxy-3-[4-[[(2-hydroxyethyl)[2-(1*H*-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide] (Ellis et al., 2009), panobinostat (Ellis et al., 2009), and OSU-HDAC42 [or AR42, (*S*)-(+)-*N*-hydroxy-4-(3-methyl-2-phenyl-butyrylamino)-benzamide] (Liu et al., 2010), and tamoxifen (Bursch et al., 1996), resveratrol (Scarlatti et al., 2008), oridonin (Cheng et al., 2009a,b), temozolomide (Cheng et al., 2012a), and arsenic trioxide (Kanzawa et al., 2005). Other drugs such as the tyrosine kinase inhibitors imatinib (Ertmer et al., 2007) and dasatinib (Milano et al., 2009), proteasome inhibitors bortezomib and NPI-0052 [(1*R*,4*R*,5*S*)-4-(2-chloroethyl)-1-((*S*)-((*S*)-cyclohex-2-enyl)(hydroxy)methyl)-5-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione] (Zhu et al., 2010), antidepressants fluoxetine and maprotiline (Cloonan and Williams, 2011), farnesyltransferase inhibitor manumycin A, FTI-276 [(2*S*)-2-[[4-[[(2*R*)-2-azaniumyl-3-sulfanylpropyl]amino]-2-phenylbenzoyl]amino]-4-methylsulfanylbutanoate], and lonafarnib (Pan et al., 2008), endoplasmic reticulum stress inducers thapsigargin and tunicamycin (Cheng et al., 2013), and angiogenesis inhibitors kringle 5 and endostatin (Ramakrishnan et al., 2007) can also induce autophagy, despite their different mechanisms of action (Table 3).

B. Application of Autophagy-Based Pharmacotherapy

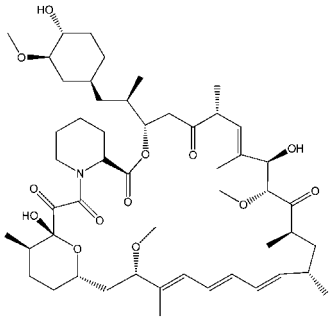
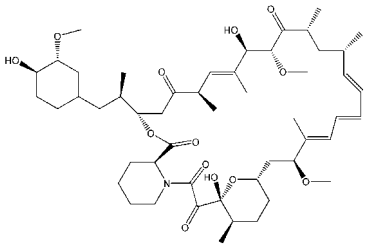
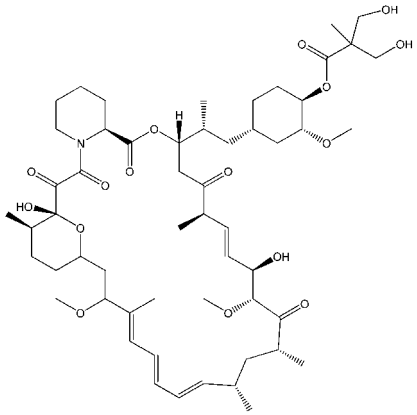
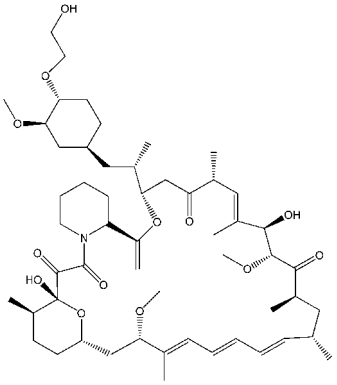
Recent advances in our understanding of the roles and functions of autophagy in human health and disease provide an exciting opportunity to target autophagy for the treatment of human illnesses (Rubinsztein et al., 2007). In the following sections, we discuss a few directions that may be of particular use in future investigations.

1. Modulation of Autophagy as a Cancer Therapy. Therapeutic targeting of the autophagy pathway as a new anticancer strategy has been under extensive investigation. Depending on the context, such as tumor type or stage, both of the autophagy-enhancing and autophagy-inhibiting agents may elicit beneficial effects in the treatment of cancer.

a. Use of autophagy inhibitors in cancer treatment. The rationale for inhibiting autophagy for cancer treatment was generated by the observation that increases in autophagy are often observed in cancer cells as an apparent attempt to maintain metabolic homeostasis and to survive external stress such as hypoxia, nutrient deprivation, chemotherapy, or radiotherapy.

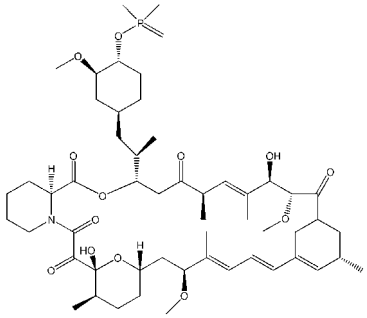
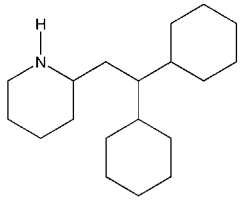
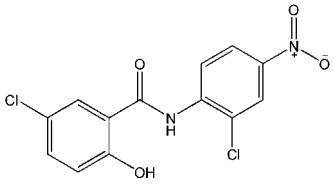
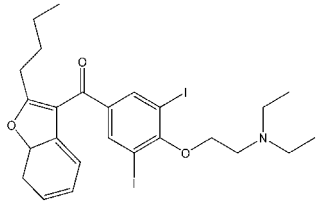
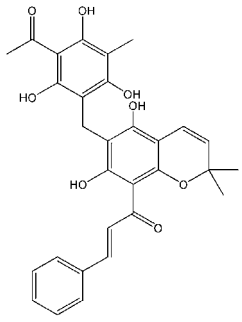
Ras-mediated transformation and tumor growth appear to use autophagy to evade metabolic stress and cell death. It was demonstrated that inhibition of autophagy by pharmacologic or genetic means suppressed K-Ras-induced growth of human breast epithelial cells in vitro and tumor growth in animal models (Kim et al., 2011b). Inhibiting autophagy using CQ or *Atg5*-targeted small-interfering RNA (siRNA) inhibited growth and tumorigenicity of pancreatic ductal carcinoma (PDAC, a tumor with K-Ras mutation) cells and in mouse xenograft models (Yang et al., 2011a). These preclinical results suggest that autophagy might be exploited as a new therapeutic target in the setting of tumors driven by oncogenic Ras, which may lead to further clinical trials using autophagy inhibitors in the patients with Ras-driven tumors. Abnormal autophagic activity was also observed in poorly differentiated non-small-cell lung cancer and was associated with extensive intratumoral

TABLE 3
Compounds known to activate autophagy

Type of Agents	Mechanism of Action	Compound	Structure
Inhibitor of mTORC1	Promotes the formation of autophagosome	Rapamycin	
		Sirolimus	
		Temsirolimus (CCI-779)	
Inhibitor of mTORC1	Promotes the formation of autophagosome	Everolimus (RAD-001)	

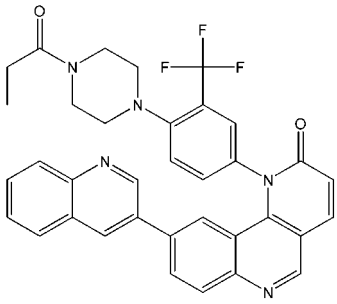
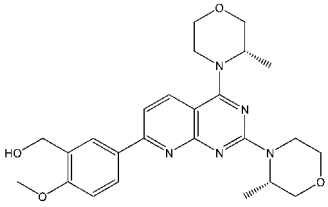
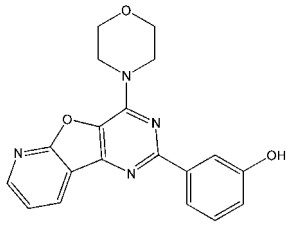
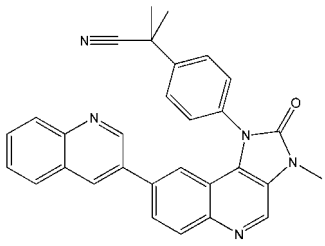
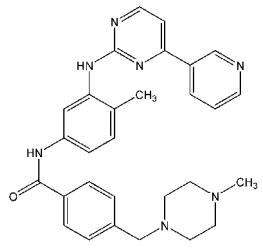
(*continued*)

TABLE 3—*Continued*

Type of Agents	Mechanism of Action	Compound	Structure
		Deforolimus (AP-23573)	
		Perhexiline	
		Niclosamide	
Inhibitor of mTORC1	Promotes the formation of autophagosome	Amiodarone	
		Rottlerin	

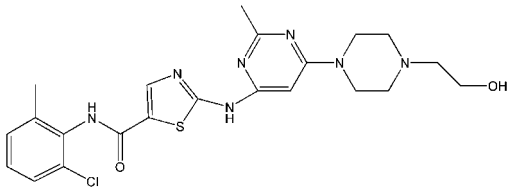
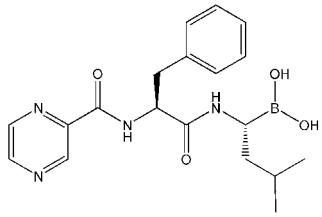
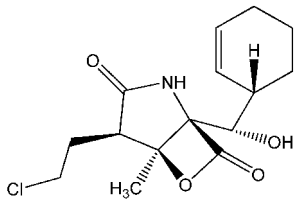
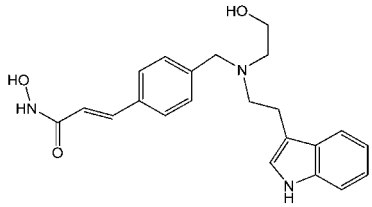
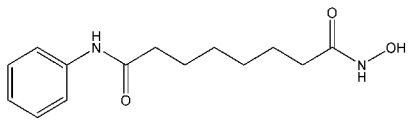
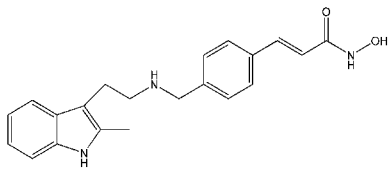
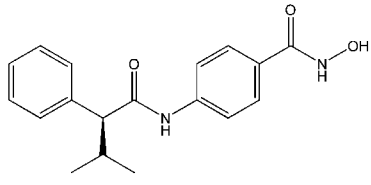
(*continued*)

TABLE 3—*Continued*

Type of Agents	Mechanism of Action	Compound	Structure
Inhibitor of mTORC1/ mTORC2	Promotes the formation of autophagosome	Torin1	
		AZD8055	
Dual inhibitor of PI3K-mTOR	Promotes the formation of autophagosome	PI-103	
		NVP-BEZ235	
Inhibitor of tyrosine kinase	Inhibits mTOR pathway	Imatinib	

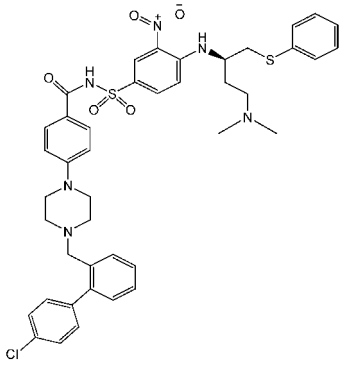
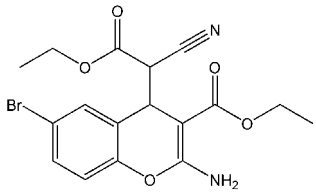
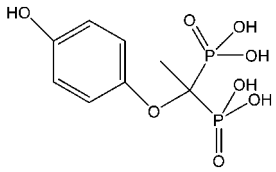
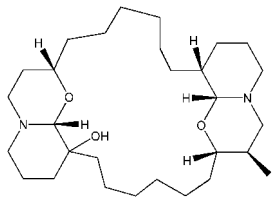
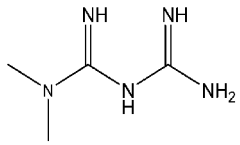
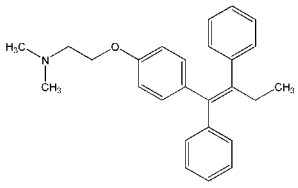
(*continued*)

TABLE 3—*Continued*

Type of Agents	Mechanism of Action	Compound	Structure
		Dasatinib	
Inhibitor of proteasome	Activates autophagy to compensate the clearance of proteins	Bortezomib	
Inhibitor of proteasome	Activates autophagy to compensate the clearance of proteins	NPI-0052	
Inhibitor of histone deacetylase	Inhibits mTOR	LAQ824	
		Vorinostat (SAHA)	
		Panobinostat	
		OSU-HDAC42	

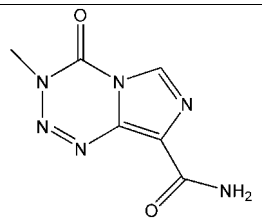
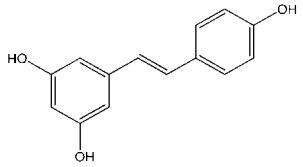
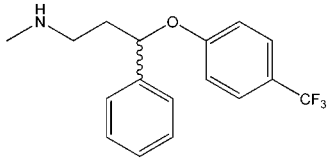
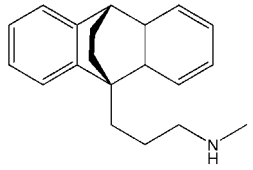
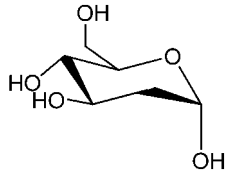
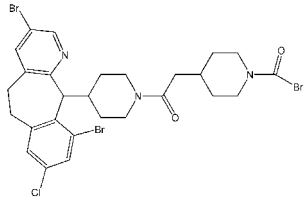
(*continued*)

TABLE 3—*Continued*

Type of Agents	Mechanism of Action	Compound	Structure
BH3 domain mimetics	Disrupts Bcl-2–Beclin1 interaction and induces autophagy	ABT-737	
		HA14-1	
Inhibitor of IP_3	Lowers inositol and IP3 levels	L-690,330	
IP3 receptor antagonist	Disrupts the molecular complex formed by the IP3 receptor and Beclin1	Xestospongin B	
Activator of AMPK	Inhibits mTOR	Metformin	
Antiestrogen	Increases Beclin1 and induces autophagy	Tamoxifen	

(*continued*)

TABLE 3—*Continued*

Type of Agents	Mechanism of Action	Compound	Structure
Alkylating agent		Temozolomide	
Antioxidant	Activates sirtuin1 and inhibits S6 kinase	Resveratrol	
Antidepressant		Fluoxetine	
		Maprotiline	
Inhibitor of glycolysis	Increases Beclin1 and induces autophagy	2-Deoxyglucose	
Inhibitor of farnesyltransferase		Lonafarnib	

necrosis, which is considered to be a poor prognostic factor (Karpathiou et al., 2011).

Autophagy is up-regulated in tumor cells subjected to various stresses, including chemotherapy and radiotherapy, and blocking autophagy may increase the effectiveness of concomitant treatments (Townsend et al., 2012), such as inhibition of autophagy by CQ-sensitized human cancer cells subjected to hypoxia and human xenografts subjected to radiation therapy (Rouschop et al., 2010). It has been shown that inhibiting autophagy enhanced the efficacy of chemotherapy and targeted therapy against cancer, and the increased efficacy was associated with increased tumor cell deaths. Suppression of autophagy via knockdown of autophagy genes or use of chemical inhibitors of autophagy such as 3-MA, CQ, or HCQ can sensitize tumor cells to the effects of chemotherapeutic drugs (Rubinsztein et al., 2012), tamoxifen (Amaravadi et al., 2007), TNF-α (Giampietri et al., 2012), proteasome inhibitors (Ding et al., 2009; Zhu et al., 2010), glycolytic inhibitor 2-deoxy-D-glucose (2-DG) (Wu et al.,

2009; Choi and Lee, 2011), tyrosine kinase inhibitors (Gupta et al., 2010), histone deacetylase (HDAC) inhibitors (Shao et al., 2004; Carew et al., 2007), and EGFR antibody and inhibitors (Cheng et al., 2010; Li and Fan, 2010).

We recently reported that blunting of autophagy by inhibiting eEF2K significantly increases the antitumor effects of Akt inhibitors such as MK-2206 [8-[4-(1-aminocyclobutyl)phenyl]-9-phenyl-1,2,4-triazolo[3,4-*f*][1,6]naphthyridin-3(2*H*)-one dihydrochloride] (Cheng et al., 2011a,b). We also found that combinatorial treatment of MK-2206 with the small molecule inhibitor of EGFR tyrosine kinase gefitinib promotes a switch from autophagy to apoptosis and enhances the efficacy of gefitinib against human glioma (Cheng et al., 2012b). The dual inhibitors of PI3K/mTOR signaling can activate cytoprotective autophagy, and inhibitors of autophagy can cooperate with the PI3K/mTOR dual inhibitors to promote tumor cell death (Fan et al., 2010). The sensitizing effects of inhibiting autophagy on the antitumor efficacy of chemotherapeutic agents have been recapitulated in preclinical models of Myc-induced lymphoma (Amaravadi et al., 2007), colon cancer (Ding et al., 2009; Carew et al., 2010; Li et al., 2010), prostate cancer (Wu et al., 2010), and glioma (Fan et al., 2010).

On the basis of the preclinical data demonstrating that targeting autophagic survival can reinforce the efficacy of anticancer therapy, a number of clinical trials have been launched to test whether the inhibitors of autophagy can benefit cancer patients (Table 4).

Of the known autophagy inhibitors, thus far only CQ and HCQ have been actively evaluated in clinical trials, partly because the pharmacologic and toxicologic profiles of these two drugs are well documented. CQ was originally introduced as a therapeutic agent for treating malaria in 1947, and it is still used as an effective and safe antimalarial drug. Furthermore, CQ is used for the treatment of rheumatoid arthritis, systemic lupus erythematosus, amoebic hepatitis, and other connective tissue disorders (Solomon and Lee, 2009). Because of retinal toxicity, a major side effect of CQ, HCQ was later developed through modification of the structure of CQ by introducing a hydroxyl group at the end of the side chain, a structural modification that results in a decreased ability to cross the blood-retinal barrier and reduces the retinal toxicity (Townsend et al., 2012). The more favorable safety profile makes HCQ preferable to CQ (Ruiz-Irastorza et al., 2010).

Early results with CQ in cancer treatment have been reported. Patients with glioblastoma were treated with surgery, chemotherapy, or radiotherapy with or without CQ (Sotelo et al., 2006). The median survival in the CQ arm was 24 months compared with 11 months in the controls (Sotelo et al., 2006). There are also several ongoing phase I/II trials evaluating the combination of HCQ or CQ with chemotherapeutic agents in patients with multiple myeloma, brain, lung, breast, colorectal, pancreas, kidney, or prostate cancers (Table 4). These results will be informative and hold the potential for increasing the interest in this field.

b. Use of autophagy activators in cancer treatment. As discussed earlier, evidence suggests that increased autophagy can kill cells. However, the weakness of many studies has been that the demonstration of autophagy after a cytotoxic treatment does not prove that autophagy contributed to cell death, only that it was associated with it. It is equally plausible that increased autophagy in these settings was a failed attempt at cell survival. To more definitively demonstrate a cytotoxic role for autophagy, inhibition would have to increase survival. In fact, most studies do not demonstrate a causative role for autophagy in promotion of cell death. Nonetheless, induction of autophagic cell death has been suggested as a potential strategy to eradicate tumor cells. In fact, several anticancer drugs have been reported to trigger cell death through autophagy-mediated mechanisms (Levy and Thorburn, 2011).

Rapamycin, a selective mTOR inhibitor, induces autophagy (Takeuchi et al., 2005), as does temsirolimus, another inhibitor of mTOR whose autophagy-inducing effect has been attributed to its antitumor mechanism in mantle cell lymphoma (Yazbeck et al., 2008). Likewise, the inhibitory effect of the mTOR inhibitor everolimus on lymphoblastic leukemia was associated with activation of autophagy (Crazzolara et al., 2009). The ATP-competitive inhibitors of mTORC1/mTORC2, WYE-125132 (WYE-132) (Yu et al., 2010), and AZD8055 (Chresta et al., 2010) have demonstrable anticancer activity against several solid tumors both in vitro and in vivo; AZD8055 is currently in phase I clinical trials. The tyrosine kinase inhibitor dasatinib has been reported to enhance the antiglioma effect of temozolomide through triggering autophagic cell death, and this action can be antagonized by the autophagy inhibitor 3-MA (Milano et al., 2009). Akt inhibitors induce a greater autophagic response and cause more cell death when combined with radiotherapy or chemotherapy (Fujiwara et al., 2007) than when used alone.

Other types of drugs possessing an autophagy-inducing effect have also found their potential application in cancer treatment. For instance, autophagic cell death may contribute to the anticancer actions of the histone deacetylase (HDAC) inhibitor vorinostat (SAHA) (Shao et al., 2004; Liu et al., 2010), the estrogen receptor antagonist tamoxifen (Bursch et al., 1996), and the antidepressants fluoxetine and maprotiline (Cloonan and Williams, 2011), as inhibition of autophagy decreases the efficacy of these agents against tumor cells. The cytotoxicity of tumor necrosis factor α (TNF-α) in fibrosarcoma cells is also associated with activation of autophagy (Cheng et al., 2008). The natural products arsenic trioxide (Kanzawa et al., 2005), resveratrol (Opipari et al., 2004), and saponin (Ellington et al., 2005) induce autophagic cell death in malignant glioma, ovarian cancer, and colon cancer, respectively. Thus, it is

TABLE 4
Clinical studies testing the effects of autophagy modulators on cancer therapies

Cancer Type	Status	Therapeutic Regimen	Autophagy Modulation	Identifier
Renal cell cancer	Phase I	HCQ	Inhibition	NCT01144169
	Phase II	Everolimus	Activation	NCT00830895
	Phase I/II	Temsirolimus + gemcitabine and cisplatin		NCT01090466
Prostate cancer	Phase II	HCQ	Inhibition	NCT00726596
	Phase II	HCQ + docetaxel		NCT00786682
	Phase II	Everolimus + pasireotide	Activation	NCT01313559
	Phase I/II	Everolimus + docetaxel and bevacizumab		NCT00574769
Breast cancer	Phase II	HCQ	Inhibition	NCT01292408
	Phase I/II	HCQ + ixabepilone		NCT00765765
	Phase I/II	CQ + tamoxifen		NCT01023477
	Phase II	Rapamycin + trastuzumab	Activation	NCT00411788
	Phase I/II	Temsirolimus + neratinib		NCT01111825
Non–small cell lung cancer	Phase II	HCQ + erlotinib	Inhibition	NCT00977470
	Phase I/II	HCQ + gefitinib		NCT00809237
	Phase I/II	HCQ + bevacizumab, carboplatin, and paclitaxel		NCT00933803
	Phase II	Temsirolimus	Activation	NCT00079235
	Phase I/II	Sirolimus + pemetrexed		NCT00923273
Small cell lung cancer	Phase I/II	CQ + radiation	Inhibition	NCT00969306
	Phase II	Everolimus	Activation	NCT00374140
	Phase I/II	Everolimus + paclitaxel		NCT01079481
Pancreatic cancer	Phase II	HCQ	Inhibition	NCT01273805
	Phase I/II	HCQ + gemcitabine		NCT01128296
	Phase I	HCQ + sunitinib		NCT00813423
	Phase II	Everolimus	Activation	NCT01648465
	Phase I	Sirolimus + vismodegib		NCT01537107
Glioblastoma	Phase I/II	HCQ + temozolomide + radiation	Inhibition	NCT00486603
	Phase III	CQ		NCT00224978
	Phase I/II	Temsirolimus + sorafenib	Activation	NCT00329719
	Phase I/II	Everolimus + temozolomide and radiation		NCT01062399
Colorectal cancer	Phase I/II	HCQ + FOLFOX + bevacizumab	Inhibition	NCT01206530
	Phase I/II	HCQ + capecitabine, oxaliplatin, and bevacizumab		NCT01006369
	Phase I/II	Everolimus + irinotecan and cetuximab	Activation	NCT00522665
	Phase I	Everolimus + OSI-906		NCT01154335
Chronic myeloid leukemia	Phase II	HCQ + imatinib	Inhibition	NCT01227135
	Phase I/II	Everolimus	Activation	NCT01188889
Chronic lymphocytic leukemia	Phase II	HCQ	Inhibition	NCT00771056
	Phase I/II	Everolimus + alemtuzumab	Activation	NCT00935792
Advanced solid tumor	Phase I	HCQ + sunitinib	Inhibition	NCT00813423
	Phase I	HCQ + temozolomide		NCT00714181
	Phase I	HCQ + temsirolimus		NCT00909831
	Phase I	HCQ + vorinostat		NC01023737
	Phase I	HCQ + sirolimus or vorinostat		NCT01266057
	Phase I	Everolimus + capecitabine, oxaliplatin, and bevacizumab	Activation	NCT00849550
	Phase I/II	Temsirolimus		NCT01020305
	Phase II	Everolimus		NCT00657982
	Phase I	Temsirolimus + vinorelbine		NCT01155258
Multiple myeloma	Phase I/II	HCQ + bortezomib	Inhibition	NCT00568880
	Phase I	HCQ + cyclophosphamide, dexamethasone, and rapamycin		NCT01689987
	Phase I	Temsirolimus + dexamethasone	Activation	NCT00693433
	Phase I	Temsirolimus + lenalidomide		NCT00398515
	Phase I/II	Everolimus + panobinostat		NCT00918333
	Phase I/II	Everolimus + sorafenib		NCT00474929
Ovarian cancer	Phase II	Temsirolimus	Activation	NCT01460979
	Phase I	Temsirolimus + pegylated liposomal doxorubicin		NCT00982631
	Phase II	Temsirolimus + carboplatin and paclitaxel		NCT01196429
	Phase II	Temsirolimus + bevacizumab		NCT01010126
	Phase II	Everolimus + bevacizumab		NCT01031381
	Phase I	Everolimus + carboplatin and pegylated liposomal doxorubicin hydrochloride		NCT01281514
Melanoma	Phase I	HCQ	Inhibition	NCT00962845
	Phase II	Temsirolimus + selumetinib	Activation	NCT01166126
	Phase II	Everolimus + paclitaxel and carboplatin		NCT01014351

FOLFOX (regimen), oxaliplatin, 5-fluorouracil, and folinic acid; OSI-906, 3-[8-amino-1-(2-phenylquinolin-7-yl)imidazo[1,5-a]pyrazin-3-yl]-1-methylcyclobutan-1-ol.

conceivable that some autophagy-inducing agents may also be useful in cancer therapies because of their ability to trigger autophagic cell death. The same attention given to inhibitors of autophagy should be given to autophagy-inducing or autophagy-enhancing agents.

2. Therapeutic Effects of Autophagy Modulators on Cardiovascular Diseases. Increasing evidence has linked autophagy with the normal function of cardiomyocytes and with the pathogenesis of multiple forms of heart disease, suggesting that components of the

autophagy pathway may include potential new targets for treating cardiac disorders. Autophagy is up-regulated in many cardiac pathologic states, exerting both protective and detrimental effects through context-dependent mechanisms. Pharmacologic modulation of autophagy may represent a new therapeutic strategy to limit the myocardial damage during cardiac stress (Table 5). So far, nine patents have disclosed the use of autophagy activators and inhibitors in the treatment of cardiovascular diseases (Nemchenko et al., 2011).

Cardiac autophagy is often activated in response to stress, and enhances the clearance of misfolded and other harmful proteins. Rapamycin, a potent activator of autophagy, has been reported to prevent cardiac hypertrophy caused by thyroid hormone or by aortic banding, and represses existing hypertrophy that results from pressure overload (Shioi et al., 2003; McMullen et al., 2004; Nishida et al., 2009). Sulfaphenazole (SUL), an antimicrobial agent, can protect the heart against I/R injury reportedly through inducing autophagy (Huang et al., 2010). Likewise, chloramphenicol succinate has been shown to activate autophagy and reduce myocardial damage during I/R (Sala-Mercado et al., 2010).

Macrophages can degrade extracellular matrix and promote the death of smooth muscle cells, thereby playing a role in atherogenesis. The clearance of macrophages from atherosclerotic plaques through autophagy has been suggested as an attractive therapeutic strategy for atherosclerosis (Martinet et al., 2007b). In line with these findings, the stent-based delivery of everolimus was shown to selectively clear macrophages from atherosclerotic plaques in rabbits by activating autophagy without altering smooth muscle cells (Verheye et al., 2007).

In other situations such as the presence of severe pressure overload, suppressing rather than activating autophagy might be beneficial. For instance, trichostatin A, an inhibitor of histone deacetylases, blunts autophagy and inhibits pathologic cardiac remodeling during severe pressure overload (Cao et al., 2011). Propofol, an antioxidant agent widely used as an anasthetic, has also been shown to inhibit autophagic cell death and limit myocardial damage during I/R injury in rats (Noh et al., 2010). Granulocyte colony-stimulating

TABLE 5
Preclinical studies of the effects of autophagy modulators on various diseases

Autophagy Modulation	Agent	Effects	Related Disease	References
Activation	Rapamycin	Prevents thyroid hormone, aortic banding, or pressure overload-induced cardiac hypertrophy	Cardiac hypertrophy	Nishida et al., 2009
	Sulfaphenazole	Protects cardiomyocytes against I/R in vitro and in vivo	Heart disease	Huang et al., 2010
	Chloramphenicol succinate	Reduces myocardial damage during I/R	Heart disease	Sala-Mercado et al., 2010
	Everolimus	Selectively clears macrophagy and protects smooth muscle cells	Atherosclerosis	Verheye et al., 2007
Inhibition	Trichostatin A	Blunts pathologic cardiac remodeling during severe pressure overload	Heart disease	Cao et al., 2011
	Propofol	Limits myocardial damage during I/R in vivo	Heart disease	Noh et al., 2010
	G-CSF	Reduces myocardial fibrosis and improves hamster survival	Cardiomyopathy	Miyata et al., 2006
	Urocortin	Reduces myocyte cell death	Heart disease	Valentim et al., 2006
Activation	Rapamycin	Promotes $CD8^+$ T cell memory formation	Infectious disease	
	AR-12	Inhibits the survival of bacteria in human and murine macrophages	Infectious disease	Chiu et al., 2009a,b
	1,25D3	Inhibits HIV replication and mycobacterial growth	Infectious disease	Campbell and Spector, 2012
Activation	Isoniazid or pyrazinamide	Dampens *M. tuberculosis*–induced proinflammatory responses in macrophagy	Infectious disease	Kim et al., 2012
Activation	Rapamycin	Clears expanded polyQ and mutant huntingtin, and reduces their toxicity in vitro and in vivo	Huntington disease	Berger et al., 2006; Ravikumar et al., 2002, 2004
	Small-molecule enhancers of rapamycin	Enhances the clearance of mutant huntingtin and α-synuclein	Huntington and Parkinson disease	Sarkar et al., 2007b
	Rapamycin	Increases the clearance of α-synuclein	Parkinson disease	Webb et al., 2003
	Lithium and trehalose	Promotes the clearance of mutant huntingtin and α-synuclein	Huntington and Parkinson disease	Sarkar et al., 2007a, 2005
	L-NAME	Enhances the degradation of huntingtin	Huntington disease	Sarkar et al., 2011
Activation	Rapamycin or methionine sulfoximine	Increases the chronologic life span in *S. cerevisiae*	Aging	Powers et al., 2006

L-NAME, N^G-nitro-L-arginine methyl ester.

factor (G-CSF) can improve the survival, cardiac function, and remodeling in the UM-X7.1 hamster model of dilated cardiomyopathy; such beneficial effects appear to be associated with a reduction in autophagic cell death (Miyata et al., 2006). Also, the reduction of autophagy in cardiac myocytes after I/R has been associated with increased cell survival (Valentim et al., 2006).

Autophagy plays a protective role in cardiomyocytes exposed to diverse forms of pathologic insults. Nutrient deprivation is the most effective inducer of autophagy in mammalian cells (Mizushima, 2007). Intermittent fasting can protect the heart from ischemic injury and attenuates cardiac remodeling after myocardial infarction, likely through the induction of autophagy; however, prolonged starvation may trigger severe cardiovascular complications and cardiac death (Martinet et al., 2007a). Thus, selection of activators or inhibitors of autophagy for prevention or treatment of cardiovascular diseases will be complicated. To date, there have been no clinical data reporting the efficacy of pharmacologic modulation of autophagy in cardiac diseases. However, it is likely that some drugs already in clinical use may have stimulatory or inhibitory effects on autophagy and these "tool" compounds may elucidate a path forward in this area.

3. Use of Autophagy Modulators for Neurologic Disorders. Several neurodegenerative diseases are characterized by accumulation of intracellular aggregate-prone proteins, including the polyQ-expanded Htt in HD, α-syn in PD, and amyloid-β in AD. Autophagy plays a critical role in degrading these misfolded and aggregate proteins. Thus, activating autophagy to facilitate the degradation of toxic proteins is a potentially novel approach to treatment of neurodegenerative disorders (Table 5).

Experiments have already shown that activation of autophagy enhances the clearance of the mutant Htt and protects against neurodegeneration in a fly model of HD (Sarkar et al., 2007b). The autophagy inducer rapamycin has been reported to promote autophagic clearance and reduce the toxicity of various polyQ proteins, including mutant Htt (Ravikumar et al., 2002; Berger et al., 2006). In the fly and mouse models of HD, rapamycin induces autophagy and protects against Htt-induced neurodegeneration (Ravikumar et al., 2004). The rapamycin analog CCI-779 decreases the formation of aggregates and lessens the symptoms of HD in a mouse model of this disease (Ravikumar et al., 2004). Rapamycin can also increase the clearance of α-syn in cells (Webb et al., 2003).

Lithium (Sarkar et al., 2005) and trehalose (Sarkar et al., 2007a) promote the clearance of both mutant Htt and α-syn via autophagic degradation through an mTOR-independent pathway. Rapamycin in combination with lithium showed a greater protection against neurodegeneration in an HD fly model as compared with either drug alone (Sarkar et al., 2008). Inhibition of nitric oxide generation by N^{G}-nitro-L-arginine methyl ester can induce autophagy, enhance the degradation of mutant Htt, and alleviate toxicity of harmful proteins in several models of HD (Sarkar et al., 2011). Combinatory use of trehalose and rapamycin has showed an additive effect on the clearance of toxic proteins and increased autophagic activity (Sarkar et al., 2007a).

These results point to the potential role of autophagy activators in the treatment of neurodegenerative diseases. To date, there are very few reported clinical results demonstrating that modulation of autophagy indeed represents an effective therapeutic intervention for these devastating diseases.

4. Autophagy Modulators for Treatment of Other Diseases.

a. Infectious and immune diseases. As described earlier, autophagy has been implicated in the host defenses against infectious agents. Therefore, it would be intriguing to determine whether targeting autophagy can serve as an effective therapeutic strategy against infectious diseases.

Several drugs that protect against microbial infections also induce autophagy (Table 5). AR-12 [2-amino-*N*-[4-[5-(2 phenanthrenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] phenyl]-acetamide], a novel small molecule inhibitor of phosphoinositide-dependent kinase-1, can induce autophagy and clear *Francisella tularensis* from the human leukemic cell line THP-1 macrophages (Chiu et al., 2009b) and *Salmonella enterica* serovar Typhimurium in murine macrophages in vitro and in vivo (Chiu et al., 2009a). Also, 1α,25-dihydroxycholecalciferol (1,25D3), the most active form of vitamin D, inhibited replication of human immunodeficiency virus (HIV) in human macrophages, and this was also associated with enhanced autophagy (Campbell and Spector, 2012). In host cells infected with *Mycobacterium tuberculosis,* induction of autophagy is correlated with the activity of antimycobacterial chemotherapy (Kim et al., 2012).

These results raise the possibility that some drugs already in clinical use for the treatment of infectious diseases exert their anti-infection effects, at least partially, via inducing autophagy. However, most of the data have resulted from in vitro and animal studies, and it is unclear whether those findings can be translated into the clinical treatment of certain infections.

Tat-beclin1, a recently developed autophagy-inducing peptide, has been shown to have potential therapeutic efficacy in the treatment of several infectious diseases (Shoji-Kawata et al., 2013). Tat-beclin1 peptide, derived from a region of beclin1, can bind human immunodeficiency virus 1 (HIV-1) and interact with a newly identified negative regulator of autophagy GAPR-1 (Golgi-associated plant pathogenesis-related protein 1, also known as GLIPR2). This study demonstrated that this autophagy-inducing peptide decreased the accumulation of polyglutamine expansion protein aggregates

and the replication of several pathogens as well as reduced mortality in mice infected with chikungunya or West Nile virus (Shoji-Kawata et al., 2013). It would be intriguing to observe the effectiveness of this autophagy-inducing peptide agent in clinical trials.

b. Aging. Aging is characterized by alterations in the disposal of damaged proteins and organelles such as mitochondria, suggesting a role for autophagy in this process (Donati, 2006). Accordingly, it has been shown that genetic disruption of TOR can extend the life span of *C. elegans* (Vellai et al., 2003). Similar observations were made in *Drosophila* (Kapahi et al., 2004). Pharmacologic inhibition of mTOR by methionine sulfoximine or rapamycin increases the life span in *Saccharomyces cerevisiae* (Powers et al., 2006). Resveratrol, a polyphenol compound, has been shown to promote longevity in several model organisms, and Sirt1-mediated autophagy is required for the life-span prolonging effects of this agent (Morselli et al., 2010). Activation of autophagy is also crucial the longevity-promoting effects of spermidine, a cationic polyamine that triggers autophagy through inhibiting histone acetyltransferases and activating various autophagy-related transcripts (Eisenberg et al., 2009). Thus, it is likely that activating autophagy may represent a new strategy for interventions in aging and that modulators of autophagy could be among the class of future antiaging agents.

V. Conclusion and Perspective

Our goal has been to provide a perspective on how autophagy-based pharmacotherapy may provide a new arena for impacting human disease. The relevant molecular mechanisms involved in autophagy regulation and their impact on numerous pathologic conditions have yielded startling, sometimes contradictory new insights. Together, the many basic, preclinical, and clinical studies suggest to us that there will be a role for modulators of autophagy in the treatment of the human illnesses. Indeed, the targeting of autophagy as a therapeutic strategy for various diseases already has begun to be explored and has shown promise.

In particular, pharmacologic approaches to the modulation of autophagy have elicited great interest. Rational searching for, designing, screening, and testing of inhibitors and activators of autophagy have become an exciting, active area within drug discovery and development, with the expectation that correcting autophagy will be a new and useful approach to the treatment of illnesses. Several drugs that affect autophagy have already been tested in animal models and in clinical trials for diseases such as cancer and cardiovascular disorders, and there are early indications of promise. Nevertheless, despite the promise of autophagy modulators as pharmacotherapeutic agents, a number of problems remain that need to be resolved before clinical modulation of autophagy in various diseases can progress. Some of these problems include the following.

1. Although we have learned a great deal about autophagy at the molecular, biochemical, and cellular levels, the precise role of autophagy in individual diseases remains incompletely understood. Many studies have been limited, showing only an association between the presence of autophagy and the effect of drugs rather than implicating autophagy directly. Future studies need to be more rigorously designed to distinguish the precise role of autophagy in disease states and in the responses to treatment. To date, translating preclinical observations into the clinic has progressed most rapidly in oncology, but major opportunities for progress exist in several other therapeutic areas, including cardiology, neuroscience, and infectious diseases and autoimmunity.
2. It is likely that in each of those disease areas there will be segments of the population more likely to respond than others, thereby requiring the identification of predictive pharmacodynamics and surrogate end-point biomarkers.
3. Most of the current drugs described as modulators of autophagy do not directly or specifically regulate the autophagy process but rather act on the pathways involved in activating or inhibiting this cellular process, which raises the question of specificity. Development of selective inhibitors or activators that act directly on the regulatory targets will rely largely on the identification and further understanding of the key targets that control autophagy and the elucidation of the molecular structure of the targets. In addition, as our sophistication in the discovery of autophagy-specific ligands increases, it may be possible to use concepts now extant in other areas of pharmacology and discover functionally selective autophagy modulators that have an appropriate signaling bias that maximizes therapeutic effects and minimizes side effects.
4. To facilitate the discovery and development of the autophagy-targeted drugs, sensitive, effective, reliable, and accurate high-throughput screening (HTS) assays would need to be established and chemical libraries enriched for new scaffolds.
5. As the modulators of autophagy (either inhibitors or activators) are expected to be administered in combination with other therapeutic agents, the possible drug-drug interactions, pharmacokinetic or pharmacodynamic, might present a potential problem that should be considered in designing and developing autophagy-targeted drugs and therapeutic regimens.

In conclusion, with the growing interest in cellular autophagy among the biomedical community, we hope that we have provided a uniquely pharmacologic point of view of the opportunities and potential applications of autophagy-targeted pharmacologic agents for therapeutic intervention in various human diseases. As a variety of human disorders have been found to be associated with changes in the activity of autophagy, this important cellular process is now considered a viable target for drug discovery, and pharmacologic modulation of autophagy as a remedy provides an unusual opportunity for exploration. Based on what we have discussed, we have reason to believe that, like the apoptosis-targeted drugs that have already shown some degree of success in clinical settings, new therapeutic agents that modulate autophagy will soon emerge and come into clinical application for the benefit of human health and that autophagy-based pharmacotherapy will become a new subject in pharmacology.

Acknowledgments

The authors thank Professor Richard Mailman for critical reading of this manuscript and insightful comments, Dr. Elizabeth M. Matthew for editing this review, and Dr. Yu Shan for help in preparing Tables 2 and 3.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Cheng, Ren, Hait, Yang.

References

Aita VM, Liang XH, Murty VV, Pincus DL, Yu W, Cayanis E, Kalachikov S, Gilliam TC, and Levine B (1999) Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. *Genomics* **59**:59–65.

Akazawa H, Komazaki S, Shimomura H, Terasaki F, Zou Y, Takano H, Nagai T, and Komuro I (2004) Diphtheria toxin-induced autophagic cardiomyocyte death plays a pathogenic role in mouse model of heart failure. *J Biol Chem* **279**: 41095–41103.

Alvers AL, Wood MS, Hu D, Kaywell AC, Dunn WA Jr, and Aris JP (2009) Autophagy is required for extension of yeast chronological life span by rapamycin. *Autophagy* **5**:847–849.

Amaravadi RK, Lippincott-Schwartz J, Yin XM, Weiss WA, Takebe N, Timmer W, DiPaola RS, Lotze MT, and White E (2011) Principles and current strategies for targeting autophagy for cancer treatment. *Clin Cancer Res* **17**:654–666.

Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, Thomas-Tikhonenko A, and Thompson CB (2007) Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *J Clin Invest* **117**: 326–336.

Arias E and Cuervo AM (2011) Chaperone-mediated autophagy in protein quality control. *Curr Opin Cell Biol* **23**:184–189.

Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, and Ogier-Denis E (2001) The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. *J Biol Chem* **276**:35243–35246.

Ashford TP and Porter KR (1962) Cytoplasmic components in hepatic cell lysosomes. *J Cell Biol* **12**:198–202.

Baehrecke EH (2002) How death shapes life during development. *Nat Rev Mol Cell Biol* **3**:779–787.

Balgi AD, Fonseca BD, Donohue E, Tsang TC, Lajoie P, Proud CG, Nabi IR, and Roberge M (2009) Screen for chemical modulators of autophagy reveals novel therapeutic inhibitors of mTORC1 signaling. *PLoS ONE* **4**:e7124.

Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouysségur J, and Mazure NM (2009) Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol* **29**: 2570–2581.

Berger Z, Ravikumar B, Menzies FM, Oroz LG, Underwood BR, Pangalos MN, Schmitt I, Wullner U, Evert BO, and O'Kane CJ, et al. (2006) Rapamycin alleviates toxicity of different aggregate-prone proteins. *Hum Mol Genet* **15**:433–442.

Bergmann A (2007) Autophagy and cell death: no longer at odds. *Cell* **131**:1032–1034.

Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, and Partridge L (2010) Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster. *Cell Metab* **11**:35–46.

Boya P, González-Polo RA, Casares N, Perfettini JL, Dessen P, Larochette N, Métivier D, Meley D, Souquere S, and Yoshimori T, et al. (2005) Inhibition of macroautophagy triggers apoptosis. *Mol Cell Biol* **25**:1025–1040.

Browne GJ, Finn SG, and Proud CG (2004) Stimulation of the AMP-activated protein kinase leads to activation of eukaryotic elongation factor 2 kinase and to its phosphorylation at a novel site, serine 398. *J Biol Chem* **279**:12220–12231.

Browne GJ and Proud CG (2004) A novel mTOR-regulated phosphorylation site in elongation factor 2 kinase modulates the activity of the kinase and its binding to calmodulin. *Mol Cell Biol* **24**:2986–2997.

Budanov AV and Karin M (2008) p53 Target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. *Cell* **134**:451–460.

Bursch W (2001) The autophagosomal-lysosomal compartment in programmed cell death. *Cell Death Differ* **8**:569–581.

Bursch W, Ellinger A, Kienzl H, Török L, Pandey S, Sikorska M, Walker R, and Hermann RS (1996) Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy. *Carcinogenesis* **17**:1595–1607.

Campbell GR and Spector SA (2012) Vitamin D inhibits human immunodeficiency virus type 1 and *Mycobacterium tuberculosis* infection in macrophages through the induction of autophagy. *PLoS Pathog* **8**:e1002689.

Cao DJ, Wang ZV, Battiprolu PK, Jiang N, Morales CR, Kong Y, Rothermel BA, Gillette TG, and Hill JA (2011) Histone deacetylase (HDAC) inhibitors attenuate cardiac hypertrophy by suppressing autophagy. *Proc Natl Acad Sci USA* **108**: 4123–4128.

Carew JS, Medina EC, Esquivel JA 2nd, Mahalingam D, Swords R, Kelly K, Zhang H, Huang P, Mita AC, and Mita MM, et al. (2010) Autophagy inhibition enhances vorinostat-induced apoptosis via ubiquitinated protein accumulation. *J Cell Mol Med* **14**:2448–2459.

Carew JS, Nawrocki ST, Kahue CN, Zhang H, Yang C, Chung L, Houghton JA, Huang P, Giles FJ, and Cleveland JL (2007) Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-Abl-mediated drug resistance. *Blood* **110**:313–322.

Carling D, Sanders MJ, and Woods A (2008) The regulation of AMP-activated protein kinase by upstream kinases. *Int J Obes (Lond)* **32** (Suppl 4):S55–S59.

Cesari R, Martin ES, Calin GA, Pentimalli F, Bichi R, McAdams H, Trapasso F, Drusco A, Shimizu M, and Masciullo V, et al. (2003) Parkin, a gene implicated in autosomal recessive juvenile parkinsonism, is a candidate tumor suppressor gene on chromosome 6q25-q27. *Proc Natl Acad Sci USA* **100**:5956–5961.

Chen N and Debnath J (2010) Autophagy and tumorigenesis. *FEBS Lett* **584**: 1427–1435.

Cheng Y, Li H, Ren X, Niu T, Hait WN, and Yang J (2010) Cytoprotective effect of the elongation factor-2 kinase-mediated autophagy in breast cancer cells subjected to growth factor inhibition. *PLoS ONE* **5**:e9715.

Cheng Y, Qiu F, and Ikejima T (2009a) Molecular mechanisms of oridonin-induced apoptosis and autophagy in murine fibrosarcoma L929 cells. *Autophagy* **5**:430–431.

Cheng Y, Qiu F, Tashiro S, Onodera S, and Ikejima T (2008) ERK and JNK mediate TNFα-induced p53 activation in apoptotic and autophagic L929 cell death. *Biochem Biophys Res Commun* **376**:483–488.

Cheng Y, Qiu F, Ye YC, Guo ZM, Tashiro S, Onodera S, and Ikejima T (2009b) Autophagy inhibits reactive oxygen species-mediated apoptosis via activating p38-nuclear factor-kappa B survival pathways in oridonin-treated murine fibrosarcoma L929 cells. *FEBS J* **276**:1291–1306.

Cheng Y, Ren X, Zhang Y, Patel R, Sharma A, Wu H, Robertson GP, Yan L, Rubin E, and Yang JM (2011a) eEF-2 kinase dictates cross-talk between autophagy and apoptosis induced by Akt Inhibition, thereby modulating cytotoxicity of novel Akt inhibitor MK-2206. *Cancer Res* **71**:2654–2663.

Cheng Y, Ren X, Zhang Y, Shan Y, Huber-Keener KJ, Zhang L, Kimball SR, Harvey H, Jefferson LS, and Yang JM (2013) Integrated regulation of autophagy and apoptosis by EEF2K controls cellular fate and modulates the efficacy of curcumin and velcade against tumor cells. *Autophagy* **9**:208–219.

Cheng Y, Sk UH, Zhang Y, Ren X, Zhang L, Huber-Keener KJ, Sun YW, Liao J, Amin S, and Sharma AK, et al. (2012a) Rational incorporation of selenium into temozolomide elicits superior antitumor activity associated with both apoptotic and autophagic cell death. *PLoS ONE* **7**:e35104.

Cheng Y, Yan L, Ren X, and Yang JM (2011b) eEF-2 kinase, another meddler in the "yin and yang" of Akt-mediated cell fate? *Autophagy* **7**:660–661.

Cheng Y, Zhang Y, Zhang L, Ren X, Huber-Keener KJ, Liu X, Zhou L, Liao J, Keihack H, and Yan L, et al. (2012b) MK-2206, a novel allosteric inhibitor of Akt, synergizes with gefitinib against malignant glioma via modulating both autophagy and apoptosis. *Mol Cancer Ther* **11**:154–164.

Chiang HL, Terlecky SR, Plant CP, and Dice JF (1989) A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. *Science* **246**: 382–385.

Chiu HC, Kulp SK, Soni S, Wang D, Gunn JS, Schlesinger LS, and Chen CS (2009a) Eradication of intracellular *Salmonella enterica* serovar Typhimurium with a small-molecule, host cell-directed agent. *Antimicrob Agents Chemother* **53**: 5236–5244.

Chiu HC, Soni S, Kulp SK, Curry H, Wang D, Gunn JS, Schlesinger LS, and Chen CS (2009b) Eradication of intracellular *Francisella tularensis* in THP-1 human macrophages with a novel autophagy inducing agent. *J Biomed Sci* **16**:110.

Choi Y and Lee JH (2011) The combination of tephrosin with 2-deoxy-D-glucose enhances the cytotoxicity via accelerating ATP depletion and blunting autophagy in human cancer cells. *Cancer Biol Ther* **12**:989–996.

Chresta CM, Davies BR, Hickson I, Harding T, Cosulich S, Critchlow SE, Vincent JP, Ellston R, Jones D, and Sini P, et al. (2010) AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. *Cancer Res* **70**:288–298.

Cloonan SM and Williams DC (2011) The antidepressants maprotiline and fluoxetine induce type II autophagic cell death in drug-resistant Burkitt's lymphoma. *Int J Cancer* **128**:1712–1723.

Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, and Kemnitz JW, et al. (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* **325**: 201–204.

Crazzolara R, Cisterne A, Thien M, Hewson J, Baraz R, Bradstock KF, and Bendall LJ (2009) Potentiating effects of RAD001 (Everolimus) on vincristine therapy in childhood acute lymphoblastic leukemia. *Blood* **113**:3297–3306.

Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, Gasco M, Garrone O, Crook T, and Ryan KM (2006) DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* **126**:121–134.

Criollo A, Maiuri MC, Tasdemir E, Vitale I, Fiebig AA, Andrews D, Molgó J, Díaz J, Lavandero S, and Harper F, et al. (2007) Regulation of autophagy by the inositol trisphosphate receptor. *Cell Death Differ* **14**:1029–1039.

Cuervo AM (2008) Autophagy and aging: keeping that old broom working. *Trends Genet* **24**:604–612.

Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, Mukherjee C, Shi Y, Gélinas C, and Fan Y, et al. (2006) Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* **10**:51–64.

Delgado MA, Elmaoued RA, Davis AS, Kyei G, and Deretic V (2008) Toll-like receptors control autophagy. *EMBO J* **27**:1110–1121.

Deretic V (2005) Autophagy in innate and adaptive immunity. *Trends Immunol* **26**: 523–528.

Deretic V (2010) Autophagy in infection. *Curr Opin Cell Biol* **22**:252–262.

Deretic V and Levine B (2009) Autophagy, immunity, and microbial adaptations. *Cell Host Microbe* **5**:527–549.

Dice JF (1990) Peptide sequences that target cytosolic proteins for lysosomal proteolysis. *Trends Biochem Sci* **15**:305–309.

Ding WX, Ni HM, Gao W, Chen X, Kang JH, Stolz DB, Liu J, and Yin XM (2009) Oncogenic transformation confers a selective susceptibility to the combined suppression of the proteasome and autophagy. *Mol Cancer Ther* **8**:2036–2045.

Ding WX and Yin XM (2012) Mitophagy: mechanisms, pathophysiological roles, and analysis. *Biol Chem* **393**:547–564.

Djavaheri-Mergny M, Amelotti M, Mathieu J, Besançon F, Bauvy C, Souquère S, Pierron G, and Codogno P (2006) NF-κB activation represses tumor necrosis factor-α-induced autophagy. *J Biol Chem* **281**:30373–30382.

Donati A (2006) The involvement of macroautophagy in aging and anti-aging interventions. *Mol Aspects Med* **27**:455–470.

Dorn BR, Dunn WA Jr, and Progulske-Fox A (2002) Bacterial interactions with the autophagic pathway. *Cell Microbiol* **4**:1–10.

Ebato C, Uchida T, Arakawa M, Komatsu M, Ueno T, Komiya K, Azuma K, Hirose T, Tanaka K, and Kominami E, et al. (2008) Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. *Cell Metab* **8**:325–332.

Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, and Taylor R, et al. (2011) Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* **331**:456–461.

Eisenberg T, Knauer H, Schauer A, Büttner S, Ruckenstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, and Antonacci L, et al. (2009) Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* **11**:1305–1314.

Ellington AA, Berhow M, and Singletary KW (2005) Induction of macroautophagy in human colon cancer cells by soybean B-group triterpenoid saponins. *Carcinogenesis* **26**:159–167.

Ellis L, Bots M, Lindemann RK, Bolden JE, Newbold A, Cluse LA, Scott CL, Strasser A, Atadja P, and Lowe SW, et al. (2009) The histone deacetylase inhibitors LAQ824 and LBH589 do not require death receptor signaling or a functional apoptosome to mediate tumor cell death or therapeutic efficacy. *Blood* **114**:380–393.

Elmore SP, Qian T, Grissom SF, and Lemasters JJ (2001) The mitochondrial permeability transition initiates autophagy in rat hepatocytes. *FASEB J* **15**: 2286–2287.

English L, Chemali M, Duron J, Rondeau C, Laplante A, Gingras D, Alexander D, Leib D, Norbury C, and Lippé R, et al. (2009) Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. *Nat Immunol* **10**:480–487.

Ertmer A, Huber V, Gilch S, Yoshimori T, Erfle V, Duyster J, Elsässer HP, and Schätzl HM (2007) The anticancer drug imatinib induces cellular autophagy. *Leukemia* **21**:936–942.

Fabri M, Realegeno SE, Jo EK, and Modlin RL (2011) Role of autophagy in the host response to microbial infection and potential for therapy. *Curr Opin Immunol* **23**: 65–70.

Fan QW, Cheng C, Hackett C, Feldman M, Houseman BT, Nicolaides T, Haas-Kogan D, James CD, Oakes SA, and Debnath J, et al. (2010) Akt and autophagy cooperate to promote survival of drug-resistant glioma. *Sci Signal* **3**:ra81.

Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, and Shokat KM (2009) Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. *PLoS Biol* **7**:e38.

Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R, Corazzari M, Fuoco C, Ucar A, and Schwartz P, et al. (2007) Ambra1 regulates autophagy and development of the nervous system. *Nature* **447**:1121–1125.

Fujiwara K, Iwado E, Mills GB, Sawaya R, Kondo S, and Kondo Y (2007) Akt inhibitor shows anticancer and radiosensitizing effects in malignant glioma cells by inducing autophagy. *Int J Oncol* **31**:753–760.

Ganley IG, Lam H, Wang J, Ding X, Chen S, and Jiang X (2009) ULK1.ATG13. FIP200 complex mediates mTOR signaling and is essential for autophagy. *J Biol Chem* **284**:12297–12305.

Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, and Springer W (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* **12**:119–131.

Gewirtz DA (2009) Autophagy, senescence and tumor dormancy in cancer therapy. *Autophagy* **5**:1232–1234.

Giampietri C, Petrungaro S, Padula F, D'Alessio A, Marini ES, Facchiano A, Filippini A, and Ziparo E (2012) Autophagy modulators sensitize prostate epithelial cancer cell lines to TNF-alpha-dependent apoptosis. *Apoptosis* **17**:1210–1222.

Goldman S, Zhang Y, and Jin S (2010) Autophagy and adipogenesis: implications in obesity and type II diabetes. *Autophagy* **6**:179–181.

Gozuacik D and Kimchi A (2004) Autophagy as a cell death and tumor suppressor mechanism. *Oncogene* **23**:2891–2906.

Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, Kamphorst JJ, Chen G, Lemons JM, and Karantza V, et al. (2011) Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev* **25**:460–470.

Gupta A, Roy S, Lazar AJ, Wang WL, McAuliffe JC, Reynoso D, McMahon J, Taguchi T, Floris G, and Debiec-Rychter M, et al. (2010) Autophagy inhibition and antimalarials promote cell death in gastrointestinal stromal tumor (GIST). *Proc Natl Acad Sci USA* **107**:14333–14338.

Gustafsson AB and Gottlieb RA (2009) Autophagy in ischemic heart disease. *Circ Res* **104**:150–158.

Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, and Shaw RJ (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* **30**:214–226.

Hamacher-Brady A, Brady NR, and Gottlieb RA (2006) Enhancing macroautophagy protects against ischemia/reperfusion injury in cardiac myocytes. *J Biol Chem* **281**: 29776–29787.

Hamacher-Brady A, Brady NR, Logue SE, Sayen MR, Jinno M, Kirshenbaum LA, Gottlieb RA, and Gustafsson AB (2007) Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy. *Cell Death Differ* **14**:146–157.

Han J, Hou W, Goldstein LA, Lu C, Stolz DB, Yin XM, and Rabinowich H (2008) Involvement of protective autophagy in TRAIL resistance of apoptosis-defective tumor cells. *J Biol Chem* **283**:19665–19677.

Hanaoka H, Noda T, Shirano Y, Kato T, Hayashi H, Shibata D, Tabata S, and Ohsumi Y (2002) Leaf senescence and starvation-induced chlorosis are accelerated by the disruption of an Arabidopsis autophagy gene. *Plant Physiol* **129**:1181–1193.

Hara T and Mizushima N (2009) Role of ULK-FIP200 complex in mammalian autophagy: FIP200, a counterpart of yeast Atg17? *Autophagy* **5**:85–87.

Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, and Okano H, et al. (2006) Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* **441**: 885–889.

Hardie DG (2011) AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. *Genes Dev* **25**:1895–1908.

Harris J, De Haro SA, Master SS, Keane J, Roberts EA, Delgado M, and Deretic V (2007) T helper 2 cytokines inhibit autophagic control of intracellular *Mycobacterium tuberculosis*. *Immunity* **27**:505–517.

Hay N and Sonenberg N (2004) Upstream and downstream of mTOR. *Genes Dev* **18**: 1926–1945.

Herrero-Martín G, Høyer-Hansen M, García-García C, Fumarola C, Farkas T, López-Rivas A, and Jäättelä M (2009) TAK1 activates AMPK-dependent cytoprotective autophagy in TRAIL-treated epithelial cells. *EMBO J* **28**:677–685.

Hershko A and Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* **67**: 425–479.

Hickson-Bick DL, Jones C, and Buja LM (2008) Stimulation of mitochondrial biogenesis and autophagy by lipopolysaccharide in the neonatal rat cardiomyocyte protects against programmed cell death. *J Mol Cell Cardiol* **44**:411–418.

Horman S, Browne G, Krause U, Patel J, Vertommen D, Bertrand L, Lavoinne A, Hue L, Proud C, and Rider M (2002) Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. *Curr Biol* **12**:1419–1423.

Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, Iemura S, Natsume T, Takehana K, and Yamada N, et al. (2009) Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* **20**:1981–1991.

Huang C, Andres AM, Ratliff EP, Hernandez G, Lee P, and Gottlieb RA (2011) Preconditioning involves selective mitophagy mediated by Parkin and p62/SQSTM1. *PLoS ONE* **6**:e20975.

Huang C, Liu W, Perry CN, Yitzhaki S, Lee Y, Yuan H, Tsukada YT, Hamacher-Brady A, Mentzer RM Jr, and Gottlieb RA (2010) Autophagy and protein kinase C are required for cardioprotection by sulfaphenazole. *Am J Physiol Heart Circ Physiol* **298**:H570–H579.

Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, Mizushima N, Tanida I, Kominami E, and Ohsumi M, et al. (2000) A ubiquitin-like system mediates protein lipidation. *Nature* **408**:488–492.

Inbal B, Bialik S, Sabanay I, Shani G, and Kimchi A (2002) DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. *J Cell Biol* **157**:455–468.

Inoki K, Zhu T, and Guan KL (2003) TSC2 mediates cellular energy response to control cell growth and survival. *Cell* **115**:577–590.

Itakura E, Kishi C, Inoue K, and Mizushima N (2008) Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol Biol Cell* **19**:5360–5372.

Janda E, Isidoro C, Carresi C, and Mollace V (2012) Defective autophagy in Parkinson's disease: role of oxidative stress. *Mol Neurobiol* **46**:639–661.

Janku F, McConkey DJ, Hong DS, and Kurzrock R (2011) Autophagy as a target for anticancer therapy. *Nat Rev Clin Oncol* **8**:528–539.

Jia K, Hart AC, and Levine B (2007) Autophagy genes protect against disease caused by polyglutamine expansion proteins in *Caenorhabditis elegans*. *Autophagy* **3**:21–25.

Jia K and Levine B (2007) Autophagy is required for dietary restriction-mediated life span extension in *C. elegans*. *Autophagy* **3**:597–599.

Jia K, Thomas C, Akbar M, Sun Q, Adams-Huet B, Gilpin C, and Levine B (2009) Autophagy genes protect against *Salmonella typhimurium* infection and mediate insulin signaling-regulated pathogen resistance. *Proc Natl Acad Sci USA* **106**: 14564–14569.

Jin S (2006) Autophagy, mitochondrial quality control, and oncogenesis. *Autophagy* **2**: 80–84.
Johnson SC, Rabinovitch PS, and Kaeberlein M (2013) mTOR is a key modulator of ageing and age-related disease. *Nature* **493**:338–345.
Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, Kundu M, and Kim DH (2009) ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* **20**:1992–2003.
Jung HS, Chung KW, Won Kim J, Kim J, Komatsu M, Tanaka K, Nguyen YH, Kang TM, Yoon KH, and Kim JW, et al. (2008) Loss of autophagy diminishes pancreatic beta cell mass and function with resultant hyperglycemia. *Cell Metab* **8**:318–324.
Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y, and Yoshimori T (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* **19**: 5720–5728.
Kabeya Y, Mizushima N, Yamamoto A, Oshitani-Okamoto S, Ohsumi Y, and Yoshimori T (2004) LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J Cell Sci* **117**:2805–2812.
Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, and Ohsumi Y (2000) Tor-mediated induction of autophagy via an Apg1 protein kinase complex. *J Cell Biol* **150**:1507–1513.
Kamada Y, Yoshino K, Kondo C, Kawamata T, Oshiro N, Yonezawa K, and Ohsumi Y (2010) Tor directly controls the Atg1 kinase complex to regulate autophagy. *Mol Cell Biol* **30**:1049–1058.
Kanki T and Klionsky DJ (2008) Mitophagy in yeast occurs through a selective mechanism. *J Biol Chem* **283**:32386–32393.
Kanki T, Wang K, Cao Y, Baba M, and Klionsky DJ (2009) Atg32 is a mitochondrial protein that confers selectivity during mitophagy. *Dev Cell* **17**:98–109.
Kanzawa T, Kondo Y, Ito H, Kondo S, and Germano I (2003) Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. *Cancer Res* **63**:2103–2108.
Kanzawa T, Zhang L, Xiao L, Germano IM, Kondo Y, and Kondo S (2005) Arsenic trioxide induces autophagic cell death in malignant glioma cells by upregulation of mitochondrial cell death protein BNIP3. *Oncogene* **24**:980–991.
Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, and Benzer S (2004) Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. *Curr Biol* **14**:885–890.
Karpathiou G, Sivridis E, Koukourakis MI, Mikroulis D, Bouros D, Froudarakis ME, and Giatromanolaki A (2011) Light-chain 3A autophagic activity and prognostic significance in non-small cell lung carcinomas. *Chest* **140**:127–134.
Kaushik S and Cuervo AM (2012) Chaperone-mediated autophagy: a unique way to enter the lysosome world. *Trends Cell Biol* **22**:407–417.
Kessel D and Reiners JJ Jr (2007) Initiation of apoptosis and autophagy by the Bcl-2 antagonist HA14-1. *Cancer Lett* **249**:294–299.
Kim J, Kundu M, Viollet B, and Guan KL (2011a) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* **13**:132–141.
Kim JJ, Lee HM, Shin DM, Kim W, Yuk JM, Jin HS, Lee SH, Cha GH, Kim JM, and Lee ZW, et al. (2012) Host cell autophagy activated by antibiotics is required for their effective antimycobacterial drug action. *Cell Host Microbe* **11**:457–468.
Kim MJ, Woo SJ, Yoon CH, Lee JS, An S, Choi YH, Hwang SG, Yoon G, and Lee SJ (2011b) Involvement of autophagy in oncogenic K-Ras-induced malignant cell transformation. *J Biol Chem* **286**:12924–12932.
Kirkegaard K, Taylor MP, and Jackson WT (2004) Cellular autophagy: surrender, avoidance and subversion by microorganisms. *Nat Rev Microbiol* **2**:301–314.
Kisen GO, Tessitore L, Costelli P, Gordon PB, Schwarze PE, Baccino FM, and Seglen PO (1993) Reduced autophagic activity in primary rat hepatocellular carcinoma and ascites hepatoma cells. *Carcinogenesis* **14**:2501–2505.
Klionsky DJ, Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, Baba M, Baehrecke EH, Bahr BA, and Ballabio A, et al. (2008) Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy* **4**:151–175.
Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, and Kominami E, et al. (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* **441**:880–884.
Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, and Uchiyama Y, et al. (2005) Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J Cell Biol* **169**:425–434.
Kondo Y, Kanzawa T, Sawaya R, and Kondo S (2005) The role of autophagy in cancer development and response to therapy. *Nat Rev Cancer* **5**:726–734.
Kraft C, Kijanska M, Kalie E, Siergiejuk E, Lee SS, Semplicio G, Stoffel I, Brezovich A, Verma M, and Hansmann I, et al. (2012) Binding of the Atg1/ULK1 kinase to the ubiquitin-like protein Atg8 regulates autophagy. *EMBO J* **31**:3691–3703.
Kroemer G and Levine B (2008) Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* **9**:1004–1010.
Kuballa P, Nolte WM, Castoreno AB, and Xavier RJ (2012) Autophagy and the immune system. *Annu Rev Immunol* **30**:611–646.
Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, and Mizushima N (2004) The role of autophagy during the early neonatal starvation period. *Nature* **432**:1032–1036.
Lavallard VJ, Meijer AJ, Codogno P, and Gual P (2012) Autophagy, signaling and obesity. *Pharmacol Res* **66**:513–525.
Lee HK, Lund JM, Ramanathan B, Mizushima N, and Iwasaki A (2007) Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science* **315**: 1398–1401.
Leu JI, Pimkina J, Frank A, Murphy ME, and George DL (2009) A small molecule inhibitor of inducible heat shock protein 70. *Mol Cell* **36**:15–27.
Levine B (2005) Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. *Cell* **120**:159–162.
Levine B and Deretic V (2007) Unveiling the roles of autophagy in innate and adaptive immunity. *Nat Rev Immunol* **7**:767–777.
Levine B and Klionsky DJ (2004) Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev Cell* **6**:463–477.
Levine B and Kroemer G (2008) Autophagy in the pathogenesis of disease. *Cell* **132**: 27–42.
Levine B, Mizushima N, and Virgin HW (2011) Autophagy in immunity and inflammation. *Nature* **469**:323–335.
Levine B and Yuan J (2005) Autophagy in cell death: an innocent convict? *J Clin Invest* **115**:2679–2688.
Levy JM and Thorburn A (2011) Targeting autophagy during cancer therapy to improve clinical outcomes. *Pharmacol Ther* **131**:130–141.
Li C, Capan E, Zhao Y, Zhao J, Stolz D, Watkins SC, Jin S, and Lu B (2006) Autophagy is induced in $CD4^+$ T cells and important for the growth factor-withdrawal cell death. *J Immunol* **177**:5163–5168.
Li J, Hou N, Faried A, Tsutsumi S, and Kuwano H (2010) Inhibition of autophagy augments 5-fluorouracil chemotherapy in human colon cancer in vitro and in vivo model. *Eur J Cancer* **46**:1900–1909.
Li X and Fan Z (2010) The epidermal growth factor receptor antibody cetuximab induces autophagy in cancer cells by downregulating HIF-1α and Bcl-2 and activating the beclin 1/hVps34 complex. *Cancer Res* **70**:5942–5952.
Li X, He L, Che KH, Funderburk SF, Pan L, Pan N, Zhang M, Yue Z, and Zhao Y (2012) Imperfect interface of Beclin1 coiled-coil domain regulates homodimer and heterodimer formation with Atg14L and UVRAG. *Nat Commun* **3**:662.
Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH, and Jung JU (2006) Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* **8**:688–699.
Liang J, Shao SH, Xu ZX, Hennessy B, Ding Z, Larrea M, Kondo S, Dumont DJ, Gutterman JU, and Walker CL, et al. (2007) The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis. *Nat Cell Biol* **9**:218–224.
Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, and Levine B (1999) Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* **402**:672–676.
Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, Herman B, and Levine B (1998) Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *J Virol* **72**:8586–8596.
Liu B, Cheng Y, and Yang JM (2013) Drug discovery in the autophagy pathways, in *Autophagy in Health and Disease* (Gottlieb RA ed) pp 161–180, Academic Press, San Diego.
Liu J, Xia H, Kim M, Xu L, Li Y, Zhang L, Cai Y, Norberg HV, Zhang T, and Furuya T, et al. (2011) Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Cell* **147**:223–234.
Liu YL, Yang PM, Shun CT, Wu MS, Weng JR, and Chen CC (2010) Autophagy potentiates the anti-cancer effects of the histone deacetylase inhibitors in hepatocellular carcinoma. *Autophagy* **6**:1057–1065.
Lock R, Roy S, Kenific CM, Su JS, Salas E, Ronen SM, and Debnath J (2011) Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. *Mol Biol Cell* **22**:165–178.
Lu Z, Luo RZ, Lu Y, Zhang X, Yu Q, Khare S, Kondo S, Kondo Y, Yu Y, and Mills GB, et al. (2008) The tumor suppressor gene ARHI regulates autophagy and tumor dormancy in human ovarian cancer cells. *J Clin Invest* **118**:3917–3929.
Luciani A, Villella VR, Esposito S, Brunetti-Pierri N, Medina D, Settembre C, Gavina M, Pulze L, Giardino I, and Pettoello-Mantovani M, et al. (2010) Defective CFTR induces aggresome formation and lung inflammation in cystic fibrosis through ROS-mediated autophagy inhibition. *Nat Cell Biol* **12**:863–875.
Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, and Thompson CB (2005) Growth factor regulation of autophagy and cell survival in the absence of apoptosis. *Cell* **120**:237–248.
Mack HI, Zheng B, Asara JM, and Thomas SM (2012) AMPK-dependent phosphorylation of ULK1 regulates ATG9 localization. *Autophagy* **8**:1197–1214.
Maiuri MC, Le Toumelin G, Criollo A, Rain JC, Gautier F, Juin P, Tasdemir E, Pierron G, Troulinaki K, and Tavernarakis N, et al. (2007a) Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. *EMBO J* **26**:2527–2539.
Maiuri MC, Malik SA, Morselli E, Kepp O, Criollo A, Mouchel PL, Carnuccio R, and Kroemer G (2009a) Stimulation of autophagy by the p53 target gene Sestrin2. *Cell Cycle* **8**:1571–1576.
Maiuri MC, Tasdemir E, Criollo A, Morselli E, Vicencio JM, Carnuccio R, and Kroemer G (2009b) Control of autophagy by oncogenes and tumor suppressor genes. *Cell Death Differ* **16**:87–93.
Maiuri MC, Zalckvar E, Kimchi A, and Kroemer G (2007b) Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* **8**:741–752.
Mancias JD and Kimmelman AC (2011) Targeting autophagy addiction in cancer. *Oncotarget* **2**:1302–1306.
Mariño G and López-Otín C (2004) Autophagy: molecular mechanisms, physiological functions and relevance in human pathology. *Cell Mol Life Sci* **61**:1439–1454.
Mariño G, Salvador-Montoliu N, Fueyo A, Knecht E, Mizushima N, and López-Otín C (2007) Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. *J Biol Chem* **282**:18573–18583.
Martinet W, Knaapen MW, Kockx MM, and De Meyer GR (2007a) Autophagy in cardiovascular disease. *Trends Mol Med* **13**:482–491.
Martinet W, Verheye S, and De Meyer GR (2007b) Selective depletion of macrophages in atherosclerotic plaques via macrophage-specific initiation of cell death. *Trends Cardiovasc Med* **17**:69–75.
Massey DC and Parkes M (2007) Genome-wide association scanning highlights two autophagy genes, ATG16L1 and IRGM, as being significantly associated with Crohn's disease. *Autophagy* **3**:649–651.
Matecic M, Smith DL, Pan X, Maqani N, Bekiranov S, Boeke JD, and Smith JS (2010) A microarray-based genetic screen for yeast chronological aging factors. *PLoS Genet* **6**:e1000921.
Mathew R, Kongara S, Beaudoin B, Karp CM, Bray K, Degenhardt K, Chen G, Jin S, and White E (2007) Autophagy suppresses tumor progression by limiting chromosomal instability. *Genes Dev* **21**:1367–1381.

Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B, and Sadoshima J (2007) Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. *Circ Res* **100**:914–922.

McMullen JR, Sherwood MC, Tarnavski O, Zhang L, Dorfman AL, Shioi T, and Izumo S (2004) Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation* **109**:3050–3055.

Meijer AJ and Codogno P (2006) Signalling and autophagy regulation in health, aging and disease. *Mol Aspects Med* **27**:411–425.

Meley D, Bauvy C, Houben-Weerts JH, Dubbelhuis PF, Helmond MT, Codogno P, and Meijer AJ (2006) AMP-activated protein kinase and the regulation of autophagic proteolysis. *J Biol Chem* **281**:34870–34879.

Milano V, Piao Y, LaFortune T, and de Groot J (2009) Dasatinib-induced autophagy is enhanced in combination with temozolomide in glioma. *Mol Cancer Ther* **8**: 394–406.

Mitchelhill KI, Michell BJ, House CM, Stapleton D, Dyck J, Gamble J, Ullrich C, Witters LA, and Kemp BE (1997) Posttranslational modifications of the 5′-AMP-activated protein kinase β1 subunit. *J Biol Chem* **272**:24475–24479.

Miyata S, Takemura G, Kawase Y, Li Y, Okada H, Maruyama R, Ushikoshi H, Esaki M, Kanamori H, and Li L, et al. (2006) Autophagic cardiomyocyte death in cardiomyopathic hamsters and its prevention by granulocyte colony-stimulating factor. *Am J Pathol* **168**:386–397.

Mizushima N (2007) Autophagy: process and function. *Genes Dev* **21**:2861–2873.

Mizushima N and Levine B (2010) Autophagy in mammalian development and differentiation. *Nat Cell Biol* **12**:823–830.

Mizushima N, Levine B, Cuervo AM, and Klionsky DJ (2008) Autophagy fights disease through cellular self-digestion. *Nature* **451**:1069–1075.

Mizushima N, Noda T, and Ohsumi Y (1999) Apg16p is required for the function of the Apg12p-Apg5p conjugate in the yeast autophagy pathway. *EMBO J* **18**: 3888–3896.

Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, Klionsky DJ, Ohsumi M, and Ohsumi Y (1998) A protein conjugation system essential for autophagy. *Nature* **395**:395–398.

Momcilovic M, Hong SP, and Carlson M (2006) Mammalian TAK1 activates Snf1 protein kinase in yeast and phosphorylates AMP-activated protein kinase in vitro. *J Biol Chem* **281**:25336–25343.

Morselli E, Maiuri MC, Markaki M, Megalou E, Pasparaki A, Palikaras K, Criollo A, Galluzzi L, Malik SA, and Vitale I, et al. (2010) Caloric restriction and resveratrol promote longevity through the Sirtuin-1-dependent induction of autophagy. *Cell Death Dis* **1**:e10.

Mortimore GE, Lardeux BR, and Adams CE (1988) Regulation of microautophagy and basal protein turnover in rat liver. Effects of short-term starvation. *J Biol Chem* **263**:2506–2512.

Münz C (2009) Enhancing immunity through autophagy. *Annu Rev Immunol* **27**: 423–449.

Nakagawa I, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, Nara A, Funao J, Nakata M, and Tsuda K, et al. (2004) Autophagy defends cells against invading group A *Streptococcus*. *Science* **306**:1037–1040.

Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, and Kim HP, et al. (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat Immunol* **12**:222–230.

Nakai A, Yamaguchi O, Takeda T, Higuchi Y, Hikoso S, Taniike M, Omiya S, Mizote I, Matsumura Y, and Asahi M, et al. (2007) The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. *Nat Med* **13**: 619–624.

Nemchenko A, Chiong M, Turer A, Lavandero S, and Hill JA (2011) Autophagy as a therapeutic target in cardiovascular disease. *J Mol Cell Cardiol* **51**:584–593.

Nishida K, Kyoi S, Yamaguchi O, Sadoshima J, and Otsu K (2009) The role of autophagy in the heart. *Cell Death Differ* **16**:31–38.

Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, Koori T, Mora M, Riggs JE, Oh SJ, and Koga Y, et al. (2000) Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). *Nature* **406**:906–910.

Noh HS, Shin IW, Ha JH, Hah YS, Baek SM, and Kim DR (2010) Propofol protects the autophagic cell death induced by the ischemia/reperfusion injury in rats. *Mol Cells* **30**:455–460.

O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, and Ludwig DL, et al. (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* **66**:1500–1508.

Okamoto K, Kondo-Okamoto N, and Ohsumi Y (2009) Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. *Dev Cell* **17**:87–97.

Onodera J and Ohsumi Y (2005) Autophagy is required for maintenance of amino acid levels and protein synthesis under nitrogen starvation. *J Biol Chem* **280**: 31582–31586.

Opipari AW Jr, Tan L, Boitano AE, Sorenson DR, Aurora A, and Liu JR (2004) Resveratrol-induced autophagocytosis in ovarian cancer cells. *Cancer Res* **64**: 696–703.

Pan J, Chen B, Su CH, Zhao R, Xu ZX, Sun L, Lee MH, and Yeung SC (2008) Autophagy induced by farnesyltransferase inhibitors in cancer cells. *Cancer Biol Ther* **7**:1679–1684.

Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Øvervatn A, Bjørkøy G, and Johansen T (2007) p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem* **282**:24131–24145.

Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, Roberts RG, Nimmo ER, Cummings FR, and Soars D, et al.; Wellcome Trust Case Control Consortium (2007) Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat Genet* **39**: 830–832.

Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, and Levine B (2005) Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* **122**:927–939.

Pattison JS and Robbins J (2011) Autophagy and proteotoxicity in cardiomyocytes. *Autophagy* **7**:1259–1260.

Pearce LR, Sommer EM, Sakamoto K, Wullschleger S, and Alessi DR (2011) Protor-1 is required for efficient mTORC2-mediated activation of SGK1 in the kidney. *Biochem J* **436**:169–179.

Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, Small S, Spencer B, Rockenstein E, and Levine B, et al. (2008) The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. *J Clin Invest* **118**:2190–2199.

Powers RW 3rd, Kaeberlein M, Caldwell SD, Kennedy BK, and Fields S (2006) Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev* **20**:174–184.

Prentice E, Jerome WG, Yoshimori T, Mizushima N, and Denison MR (2004) Coronavirus replication complex formation utilizes components of cellular autophagy. *J Biol Chem* **279**:10136–10141.

Proud CG (2007) Cell signaling. mTOR, unleashed. *Science* **318**:926–927.

Pua HH, Dzhagalov I, Chuck M, Mizushima N, and He YW (2007) A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *J Exp Med* **204**:25–31.

Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, and Ohsumi Y, et al. (2003) Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest* **112**:1809–1820.

Qu X, Zou Z, Sun Q, Luby-Phelps K, Cheng P, Hogan RN, Gilpin C, and Levine B (2007) Autophagy gene-dependent clearance of apoptotic cells during embryonic development. *Cell* **128**:931–946.

Rabinowitz JD and White E (2010) Autophagy and metabolism. *Science* **330**: 1344–1348.

Rajawat YS, Hilioti Z, and Bossis I (2009) Aging: central role for autophagy and the lysosomal degradative system. *Ageing Res Rev* **8**:199–213.

Ramakrishnan S, Nguyen TM, Subramanian IV, and Kelekar A (2007) Autophagy and angiogenesis inhibition. *Autophagy* **3**:512–515.

Ravikumar B, Duden R, and Rubinsztein DC (2002) Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Hum Mol Genet* **11**:1107–1117.

Ravikumar B, Sarkar S, Davies JE, Futter M, Garcia-Arencibia M, Green-Thompson ZW, Jimenez-Sanchez M, Korolchuk VI, Lichtenberg M, and Luo S, et al. (2010) Regulation of mammalian autophagy in physiology and pathophysiology. *Physiol Rev* **90**:1383–1435.

Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, and O'Kane CJ, et al. (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet* **36**:585–595.

Rikihisa Y (1984) Glycogen autophagosomes in polymorphonuclear leukocytes induced by rickettsiae. *Anat Rec* **208**:319–327.

Rothermel BA and Hill JA (2008) Autophagy in load-induced heart disease. *Circ Res* **103**:1363–1369.

Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, Keulers T, Mujcic H, Landuyt W, and Voncken JW, et al. (2010) The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *J Clin Invest* **120**:127–141.

Rubinsztein DC (2006) The roles of intracellular protein-degradation pathways in neurodegeneration. *Nature* **443**:780–786.

Rubinsztein DC, Codogno P, and Levine B (2012) Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat Rev Drug Discov* **11**:709–730.

Rubinsztein DC, Gestwicki JE, Murphy LO, and Klionsky DJ (2007) Potential therapeutic applications of autophagy. *Nat Rev Drug Discov* **6**:304–312.

Rubinsztein DC, Mariño G, and Kroemer G (2011) Autophagy and aging. *Cell* **146**: 682–695.

Ruiz-Irastorza G, Ramos-Casals M, Brito-Zeron P, and Khamashta MA (2010) Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review. *Ann Rheum Dis* **69**:20–28.

Sabatini DM (2006) mTOR and cancer: insights into a complex relationship. *Nat Rev Cancer* **6**:729–734.

Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, and Komatsu M, et al. (2008) Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* **456**:264–268.

Sala-Mercado JA, Wider J, Undyala VV, Jahania S, Yoo W, Mentzer RM Jr, Gottlieb RA, and Przyklenk K (2010) Profound cardioprotection with chloramphenicol succinate in the swine model of myocardial ischemia-reperfusion injury. *Circulation* **122**(Suppl)S179–S184.

Salminen A and Kaarniranta K (2009) Regulation of the aging process by autophagy. *Trends Mol Med* **15**:217–224.

Sarkar S, Davies JE, Huang Z, Tunnacliffe A, and Rubinsztein DC (2007a) Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *J Biol Chem* **282**:5641–5652.

Sarkar S, Floto RA, Berger Z, Imarisio S, Cordenier A, Pasco M, Cook LJ, and Rubinsztein DC (2005) Lithium induces autophagy by inhibiting inositol monophosphatase. *J Cell Biol* **170**:1101–1111.

Sarkar S, Korolchuk VI, Renna M, Imarisio S, Fleming A, Williams A, Garcia-Arencibia M, Rose C, Luo S, and Underwood BR, et al. (2011) Complex inhibitory effects of nitric oxide on autophagy. *Mol Cell* **43**:19–32.

Sarkar S, Krishna G, Imarisio S, Saiki S, O'Kane CJ, and Rubinsztein DC (2008) A rational mechanism for combination treatment of Huntington's disease using lithium and rapamycin. *Hum Mol Genet* **17**:170–178.

Sarkar S, Perlstein EO, Imarisio S, Pineau S, Cordenier A, Maglathlin RL, Webster JA, Lewis TA, O'Kane CJ, and Schreiber SL, et al. (2007b) Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nat Chem Biol* **3**:331–338.

Scarlatti F, Maffei R, Beau I, Codogno P, and Ghidoni R (2008) Role of non-canonical Beclin 1-independent autophagy in cell death induced by resveratrol in human breast cancer cells. *Cell Death Differ* **15**:1318–1329.
Schmelzle T and Hall MN (2000) TOR, a central controller of cell growth. *Cell* **103**: 253–262.
Schmid D and Münz C (2007) Innate and adaptive immunity through autophagy. *Immunity* **27**:11–21.
Schmid D, Pypaert M, and Münz C (2007) Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* **26**:79–92.
Shang L, Chen S, Du F, Li S, Zhao L, and Wang X (2011) Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. *Proc Natl Acad Sci USA* **108**:4788–4793.
Shang L and Wang X (2011) AMPK and mTOR coordinate the regulation of Ulk1 and mammalian autophagy initiation. *Autophagy* **7**:924–926.
Shao Y, Gao Z, Marks PA, and Jiang X (2004) Apoptotic and autophagic cell death induced by histone deacetylase inhibitors. *Proc Natl Acad Sci USA* **101**: 18030–18035.
Shaw RJ and Cantley LC (2006) Ras, PI(3)K and mTOR signalling controls tumour cell growth. *Nature* **441**:424–430.
Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, and Tsujimoto Y (2004) Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. *Nat Cell Biol* **6**:1221–1228.
Shintani T and Klionsky DJ (2004) Autophagy in health and disease: a double-edged sword. *Science* **306**:990–995.
Shioi T, McMullen JR, Tarnavski O, Converso K, Sherwood MC, Manning WJ, and Izumo S (2003) Rapamycin attenuates load-induced cardiac hypertrophy in mice. *Circulation* **107**:1664–1670.
Shoji-Kawata S, Sumpter R, Leveno M, Campbell GR, Zou Z, Kinch L, Wilkins AD, Sun Q, Pallauf K, and MacDuff D, et al. (2013) Identification of a candidate therapeutic autophagy-inducing peptide. *Nature* **494**:201–206.
Simonsen A, Cumming RC, Brech A, Isakson P, Schubert DR, and Finley KD (2008) Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult *Drosophila*. *Autophagy* **4**:176–184.
Simonsen A and Tooze SA (2009) Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. *J Cell Biol* **186**:773–782.
Solomon VR and Lee H (2009) Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies. *Eur J Pharmacol* **625**:220–233.
Sotelo J, Briceño E, and López-González MA (2006) Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* **144**:337–343.
Spencer B, Potkar R, Trejo M, Rockenstein E, Patrick C, Gindi R, Adame A, Wyss-Coray T, and Masliah E (2009) Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in alpha-synuclein models of Parkinson's and Lewy body diseases. *J Neurosci* **29**:13578–13588.
Takahashi Y, Coppola D, Matsushita N, Cualing HD, Sun M, Sato Y, Liang C, Jung JU, Cheng JQ, and Mulé JJ, et al. (2007) Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol* **9**:1142–1151.
Takahashi Y, Hori T, Cooper TK, Liao J, Desai N, Serfass JM, Young MM, Park S, Izu Y, and Wang HG (2013) Bif-1 haploinsufficiency promotes chromosomal instability and accelerates Myc-driven lymphomagenesis via suppression of mitophagy. *Blood* **121**:1622–1632.
Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K, and Mizushima N (2011) Autophagy-deficient mice develop multiple liver tumors. *Genes Dev* **25**:795–800.
Takemura G, Miyata S, Kawase Y, Okada H, Maruyama R, and Fujiwara H (2006) Autophagic degeneration and death of cardiomyocytes in heart failure. *Autophagy* **2**:212–214.
Takeuchi H, Kondo Y, Fujiwara K, Kanzawa T, Aoki H, Mills GB, and Kondo S (2005) Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. *Cancer Res* **65**: 3336–3346.
Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lüllmann-Rauch R, Janssen PM, Blanz J, von Figura K, and Saftig P (2000) Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* **406**:902–906.
Tannous P, Zhu H, Johnstone JL, Shelton JM, Rajasekaran NS, Benjamin IJ, Nguyen L, Gerard RD, Levine B, and Rothermel BA, et al. (2008) Autophagy is an adaptive response in desmin-related cardiomyopathy. *Proc Natl Acad Sci USA* **105**:9745–9750.
Tasdemir E, Maiuri MC, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M, Criollo A, Morselli E, Zhu C, and Harper F, et al. (2008) Regulation of autophagy by cytoplasmic p53. *Nat Cell Biol* **10**:676–687.
Terman A and Brunk UT (2005) Autophagy in cardiac myocyte homeostasis, aging, and pathology. *Cardiovasc Res* **68**:355–365.
Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, and Gray NS (2009) An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J Biol Chem* **284**:8023–8032.
Tóth ML, Sigmond T, Borsos E, Barna J, Erdélyi P, Takács-Vellai K, Orosz L, Kovács AL, Csikós G, and Sass M, et al. (2008) Longevity pathways converge on autophagy genes to regulate life span in *Caenorhabditis elegans*. *Autophagy* **4**:330–338.
Townsend KN, Hughson LR, Schlie K, Poon VI, Westerback A, and Lum JJ (2012) Autophagy inhibition in cancer therapy: metabolic considerations for antitumor immunity. *Immunol Rev* **249**:176–194.
Turcotte S, Chan DA, Sutphin PD, Hay MP, Denny WA, and Giaccia AJ (2008) A molecule targeting VHL-deficient renal cell carcinoma that induces autophagy. *Cancer Cell* **14**:90–102.
Valentim L, Laurence KM, Townsend PA, Carroll CJ, Soond S, Scarabelli TM, Knight RA, Latchman DS, and Stephanou A (2006) Urocortin inhibits Beclin1-mediated autophagic cell death in cardiac myocytes exposed to ischaemia/reperfusion injury. *J Mol Cell Cardiol* **40**:846–852.
Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, and Müller F (2003) Genetics: influence of TOR kinase on lifespan in *C. elegans*. *Nature* **426**:620.
Verheye S, Martinet W, Kockx MM, Knaapen MW, Salu K, Timmermans JP, Ellis JT, Kilpatrick DL, and De Meyer GR (2007) Selective clearance of macrophages in atherosclerotic plaques by autophagy. *J Am Coll Cardiol* **49**:706–715.
Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, Kim J, May J, Tocilescu MA, Liu W, and Ko HS, et al. (2010) PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc Natl Acad Sci USA* **107**:378–383.
Vousden KH and Lane DP (2007) p53 in health and disease. *Nat Rev Mol Cell Biol* **8**: 275–283.
Wang RC, Wei Y, An Z, Zou Z, Xiao G, Bhagat G, White M, Reichelt J, and Levine B (2012) Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. *Science* **338**:956–959.
Warden SM, Richardson C, O'Donnell J Jr, Stapleton D, Kemp BE, and Witters LA (2001) Post-translational modifications of the β-1 subunit of AMP-activated protein kinase affect enzyme activity and cellular localization. *Biochem J* **354**:275–283.
Webb JL, Ravikumar B, Atkins J, Skepper JN, and Rubinsztein DC (2003) Alpha-Synuclein is degraded by both autophagy and the proteasome. *J Biol Chem* **278**: 25009–25013.
Weidberg H, Shvets E, and Elazar Z (2011) Biogenesis and cargo selectivity of autophagosomes. *Annu Rev Biochem* **80**:125–156.
White E, Karp C, Strohecker AM, Guo Y, and Mathew R (2010) Role of autophagy in suppression of inflammation and cancer. *Curr Opin Cell Biol* **22**:212–217.
Woods A, Vertommen D, Neumann D, Turk R, Bayliss J, Schlattner U, Wallimann T, Carling D, and Rider MH (2003) Identification of phosphorylation sites in AMP-activated protein kinase (AMPK) for upstream AMPK kinases and study of their roles by site-directed mutagenesis. *J Biol Chem* **278**:28434–28442.
Wu H, Yang JM, Jin S, Zhang H, and Hait WN (2006) Elongation factor-2 kinase regulates autophagy in human glioblastoma cells. *Cancer Res* **66**:3015–3023.
Wu H, Zhu H, Liu DX, Niu TK, Ren X, Patel R, Hait WN, and Yang JM (2009) Silencing of elongation factor-2 kinase potentiates the effect of 2-deoxy-D-glucose against human glioma cells through blunting of autophagy. *Cancer Res* **69**:2453–2460.
Wu Z, Chang PC, Yang JC, Chu CY, Wang LY, Chen NT, Ma AH, Desai SJ, Lo SH, and Evans CP, et al. (2010) Autophagy blockade sensitizes prostate cancer cells towards Src family kinase inhibitors. *Genes Cancer* **1**:40–49.
Yan L, Vatner DE, Kim SJ, Ge H, Masurekar M, Massover WH, Yang G, Matsui Y, Sadoshima J, and Vatner SF (2005) Autophagy in chronically ischemic myocardium. *Proc Natl Acad Sci USA* **102**:13807–13812.
Yang L, Li P, Fu S, Calay ES, and Hotamisligil GS (2010) Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell Metab* **11**:467–478.
Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, Bause A, Li Y, Stommel JM, and Dell'antonio G, et al. (2011a) Pancreatic cancers require autophagy for tumor growth. *Genes Dev* **25**:717–729.
Yang Z and Klionsky DJ (2010) Eaten alive: a history of macroautophagy. *Nat Cell Biol* **12**:814–822.
Yang ZJ, Chee CE, Huang S, and Sinicrope F (2011b) Autophagy modulation for cancer therapy. *Cancer Biol Ther* **11**:169–176.
Yang ZJ, Chee CE, Huang S, and Sinicrope FA (2011c) The role of autophagy in cancer: therapeutic implications. *Mol Cancer Ther* **10**:1533–1541.
Yazbeck VY, Buglio D, Georgakis GV, Li Y, Iwado E, Romaguera JE, Kondo S, and Younes A (2008) Temsirolimus downregulates p21 without altering cyclin D1 expression and induces autophagy and synergizes with vorinostat in mantle cell lymphoma. *Exp Hematol* **36**:443–450.
Yeh YY, Shah KH, and Herman PK (2011) An Atg13 protein-mediated self-association of the Atg1 protein kinase is important for the induction of autophagy. *J Biol Chem* **286**:28931–28939.
Yorimitsu T and Klionsky DJ (2005) Autophagy: molecular machinery for self-eating. *Cell Death Differ* **12** (Suppl 2):1542–1552.
Yoshimoto K, Hanaoka H, Sato S, Kato T, Tabata S, Noda T, and Ohsumi Y (2004) Processing of ATG8s, ubiquitin-like proteins, and their deconjugation by ATG4s are essential for plant autophagy. *Plant Cell* **16**:2967–2983.
Youle RJ and Narendra DP (2011) Mechanisms of mitophagy. *Nat Rev Mol Cell Biol* **12**:9–14.
Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, Tavaré S, Arakawa S, Shimizu S, and Watt FM, et al. (2009) Autophagy mediates the mitotic senescence transition. *Genes Dev* **23**:798–803.
Yu K, Shi C, Toral-Barza L, Lucas J, Shor B, Kim JE, Zhang WG, Mahoney R, Gaydos C, and Tardio L, et al. (2010) Beyond rapalog therapy: preclinical pharmacology and antitumor activity of WYE-125132, an ATP-competitive and specific inhibitor of mTORC1 and mTORC2. *Cancer Res* **70**:621–631.
Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, Baehrecke EH, and Lenardo MJ (2004) Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. *Science* **304**:1500–1502.
Yue Z, Jin S, Yang C, Levine AJ, and Heintz N (2003) Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci USA* **100**:15077–15082.
Zhang Y, Goldman S, Baerga R, Zhao Y, Komatsu M, and Jin S (2009) Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. *Proc Natl Acad Sci USA* **106**:19860–19865.
Zhong Y, Wang QJ, Li X, Yan Y, Backer JM, Chait BT, Heintz N, and Yue Z (2009) Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. *Nat Cell Biol* **11**:468–476.
Zhou R, Yazdi AS, Menu P, and Tschopp J (2011) A role for mitochondria in NLRP3 inflammasome activation. *Nature* **469**:221–225.
Zhu H, Tannous P, Johnstone JL, Kong Y, Shelton JM, Richardson JA, Le V, Levine B, Rothermel BA, and Hill JA (2007) Cardiac autophagy is a maladaptive response to hemodynamic stress. *J Clin Invest* **117**:1782–1793.
Zhu K, Dunner K Jr, and McConkey DJ (2010) Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. *Oncogene* **29**:451–462.