Chapter 8
Autophagy and Cell Death to Target Cancer Cells: Exploiting Synthetic Lethality as Cancer Therapies

Julie Reyjal, Kevin Cormier, and Sandra Turcotte

Abstract Since 1940 chemotherapy has been one of the major therapies used to kill cancer cells. However, conventional standard cytotoxic agents have a low therapeutic index and often show toxicity in healthy cells. Over the past decade, progress in molecular biology and genomics has identified signaling pathways and mutations driving different types of cancer. Genetic and epigenetic alterations that characterize tumor cells have been used in the development of targeted therapy, a very active area of cancer research. Moreover, identification of synthetic lethal interactions between two altered genes in cancer cells shows much promise to target specifically tumor cells. For a long time, apoptosis was considered the principal mechanism by which cells die from chemotherapeutic agents. Autophagy, necroptosis (a programmed cell death mechanism of necrosis), and lysosomal-mediated cell death significantly improve our understanding of how malignancy can be targeted by anticancer treatments. Autophagy is a highly regulated process by which misfolded proteins and organelles reach lysosomes for their degradation. Alterations in this cellular process have been observed in several pathological conditions, including cancer. The role of autophagy in cancer raised a paradox wherein it can act as a tumor suppressor at early stage of tumor development but can also be used by cancer cells as cytoprotection to promote survival in established tumors. It is interesting that autophagy can be targeted by anticancer agents to provoke cancer cell death. This review focuses on the role of autophagy in cancer cells and its potential to therapeutically kill cancer cells.

Keywords Autophagy • Cancer • Cell death • Targeted therapy • Synthetic lethality • Renal cell carcinoma • von Hippel-Lindau

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8.1 Overview of the Autophagy Machinery

Autophagy is a self-digestive process. From the Greek *auto*, meaning "oneself," and *phagy*, meaning "eating," this process is highly conserved in organisms from yeast to mammals and acts to remove misfolded proteins, aggregates, lipids, and damaged organelles. To maintain cellular homeostasis, cytoplasmic cargoes are sequestered into vesicles that reach lysosomes, where the material is degraded (Yang and Klionsky 2010). There are different types of autophagy, ranging from nonselective macroautophagy to selective autophagy such as chaperone-mediated autophagy, microautophagy, and the type based on the origin of the sequestered cargo, including mitophagy for mitochondria. Chaperone-mediated autophagy targets specific proteins containing the KFERQ sequence across the lysosome membrane, whereas microautophagy involves the direct engulfment of cytoplasm at the lysosome surface by invagination of the lysosome membrane (Reggiori et al. 2012). In contrast, macroautophagy (referred to hereafter as autophagy) is mediated by the special organelle autophagosome that engulfs proteins, lipids, and damaged organelles into double-membraned vesicles. Then the autophagosome fuses with an endosome/lysosome, a single-membrane vesicle, where the cargo is degraded through lysosomal activity (Fig. 8.1) (Klionsky and Emr 2000). Autophagy is activated under physiological and pathological conditions, such as nutrient starvation, hypoxia, metabolic stress, and in response to drugs and radiation. This dynamic process generates cellular energy resources that allow a cell to adapt its metabolism to energy demand. Defects during any step of the autophagy process result in the accumulation of damaged proteins and/or genomic damage that can stimulate the development of many human diseases, including neurodegeneration, infectious disease, heart disease, and cancer (Levine and Kroemer 2008; Turcotte and Giaccia 2010).

8.1.1 Autophagosome Formation

The unique structure of the autophagosome was first observed more than 50 years ago using electronic microscopy, and successive studies have demonstrated that autophagy is regulated through activation of autophagy-related genes (Atg) (Yang and Klionsky 2010). These genes were first identified in yeast, and many of them are found as homologs in murine and human cells (Takeshige et al. 1992). More than 15 mammalian Atg proteins have been identified and regulate the formation of autophagosomes (Table 8.1) (Mizushima et al. 2011). The initiation stage of this process engages the formation of a phagophore, followed by its elongation and closure to form an autophagosome. The origin of the phagophore is still controversial, but the endoplasmic reticulum membrane (Axe et al. 2008; Hayashi-Nishino et al. 2009; Yla-Anttila et al. 2009), mitochondrial outer membrane (Hailey et al. 2010), and plasma membrane (Ravikumar et al. 2010) have been suggested to contribute to autophagosome formation.

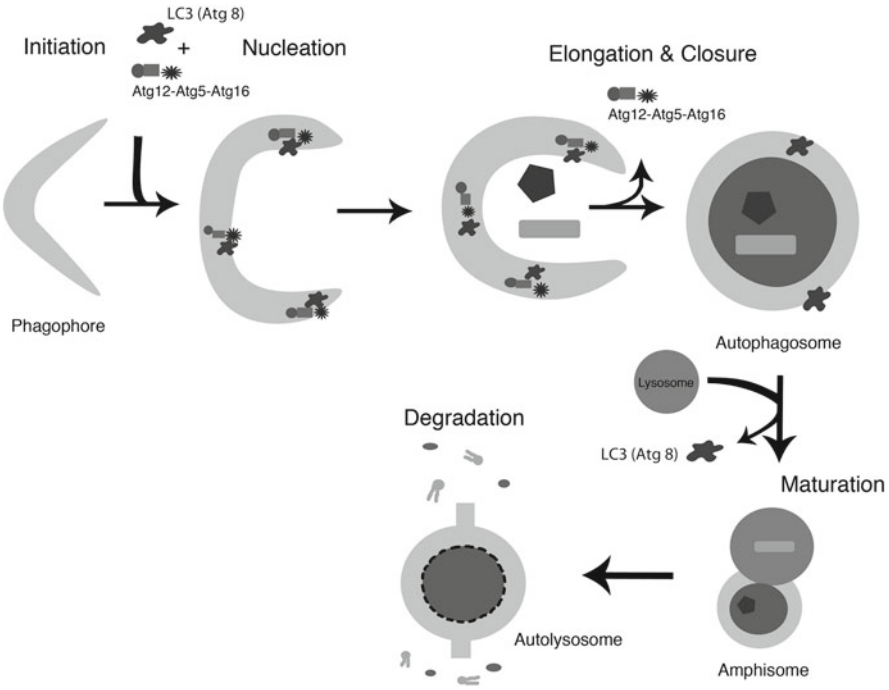

Fig. 8.1 Principal steps regulating the autophagy process. Autophagy involves the formation of double-membrane autophagosomes that fuse with lysosomes to form autolysosomes for the degradation of intracellular proteins and organelles. Under conditions of nutrient deprivation or microenvironmental stress, initiation gives rise to a phagophore, which elongates while being regulated by a series of autophagy-related genes. The phagophore closes into an autophagosome. This autophagosome then fuses with a lysosome to become an amphisome, which will mature and give rise to an autolysosome, where the encapsulated material is degraded via lysosomal activity

The activity of the autophagic machinery is regulated by different complexes: the ULK1/2 kinase complex, the vacuolar sorting protein (Vps) 34/Beclin-1 complex, the shuttling of the Atg9 protein (the only transmembrane Atg) between organelles including endosomes, and the two ubiquitin-conjugation systems, the Atg5-Atg12-Atg16 and Atg8/LC3 complexes (Fig. 8.2) (Orsi et al. 2012; Lamb et al. 2013; Rubinsztein et al. 2012). *ULK1* and *ULK2*, two Atg1 homologs, are associated with Atg13 and FIP200 in a large complex that integrates stress signals from the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) (Jung et al. 2009; Mizushima 2010). Many signals, including growth factors, amino acids, glucose, and energy status, regulate mTORC1. Upon inhibition of mTORC1 induced by starvation or chemotherapeutic agents targeting mTOR, *ULK1* and *ULK2* are phosphorylated and activated, initiating the autophagy cascade. Other complexes essential to autophagosome formation is Beclin-1, the Atg6 homolog, and Vps34, a class III phosphoinositide 3-kinase (PI3K), which recruit autophagy proteins such

Table 8.1 Autophagy-related genes involved in autophagosome formation

Yeast name	Human orthologs	Functions
Atg1	ULK1/2	Serine protein kinase
		Component of complex ULK1-FIP200-Atg13
Atg2	Atg2a	Autophagosome closure
	Atg2b	Component of complex Atg9-Atg2-Atg18
Atg3	Atg3	E2-like enzyme required for LC3 lipidation
Atg4	Atg4A, 4B, 4C, 4D	Cysteine protease involved in LC3 lipidation
Atg5	Atg5	Atg5-Atg12 ubiquitin conjugation complex
Atg6	Beclin-1	Component of the PI3K-Vps34-Beclin complex
Atg7	Atg7	E1-like enzyme activates LC3 and Atg12
Atg8	LC3A, LC3B, LC3C	Autophagosome marker, ubiquitin-like protein conjugated to phosphatidylethanolamine
	GABARAP, GABARAPL1, GABARAPL2	
Atg9	Atg9a	Transmembrane protein
	Atg9b	Component of Atg9-Atg2-Atg18 complex
Atg10	Atg10	E2-like enzyme conjugates Atg12 to Atg5
Atg12	Atg12	Ubiquitin-like protein conjugated to Atg5
Atg13	Atg13	Response to mTOR signaling
		Component of complex ULK1-Atg13-FIP200
Atg14	Atg14	Component of the PI3K-Vps34-Beclin complex
Atg16	Atg16L1	Component of Atg5-Atg12-Atg16 complex
Atg17	FIP200	Component of ULK1-Atg13-FIP200 complex
Atg18	WIP1/2	Component of Atg9-Atg2-Atg18 complex

mTOR mammalian target of rapamycin; *PI3K* phosphoinositide 3-kinase; *Vps* vacuolar sorting protein

as *UVRAG* (ultraviolet irradiation resistance-associated gene), *Ambra-1*, *Bif-1*, and *Barkor* (Kroemer et al. 2010). Furthermore, Beclin-1 binds to anti-apoptotic proteins of the BCL-2 family, such as BCL-$X_{L,}$ through a BCL-2 homology 3 domains and inhibits autophagy (Pattingre et al. 2005; Erlich et al. 2007). In response to starvation, phosphorylation on Bcl-2 by Jun kinase 1 dissociates the binding between Bcl-2 and Beclin-1 and allow Beclin-1 to induce autophagy (Wei et al. 2008; Pattingre et al. 2009). BCL-2 homology 3 mimics can also disrupt Bcl-2 and Beclin-1 binding. Finally, there are two ubiquitin conjugation systems that have been associated with autophagosome formation: Atg12-Atg5-Atg16 and Atg8/LC3. Atg5 and Atg12 were the first Atgs identified in mammals by Mizushima et al. (1998), who reported that the Atg5-Atg12-Atg16 conjugation system was conserved. The other ubiquitin conjugation system is MAP1LC3 (also called LC3), the mammalian Atg8 homolog (Kabeya et al. 2000). In unstressed cells, LC3 is present in cytoplasm in an unprocessed form, LC3I, which is converted into a phosphatidylethanolamine-conjugated form, LC3II, associated with completed autophagosomes. LC3II remains associated with the double-membraned vesicle until fusion with lysosomes. The identification of LC3 is an important finding that is routinely used to monitor autophagy in eukaryote cells. Moreover, LC3 binds the p62/sequestome1 (SQSMT1) protein via its LC3-interactin region domain and prevents its accumulation (Pankiv et al. 2007). p62 Is an adaptor protein involved in protein trafficking to

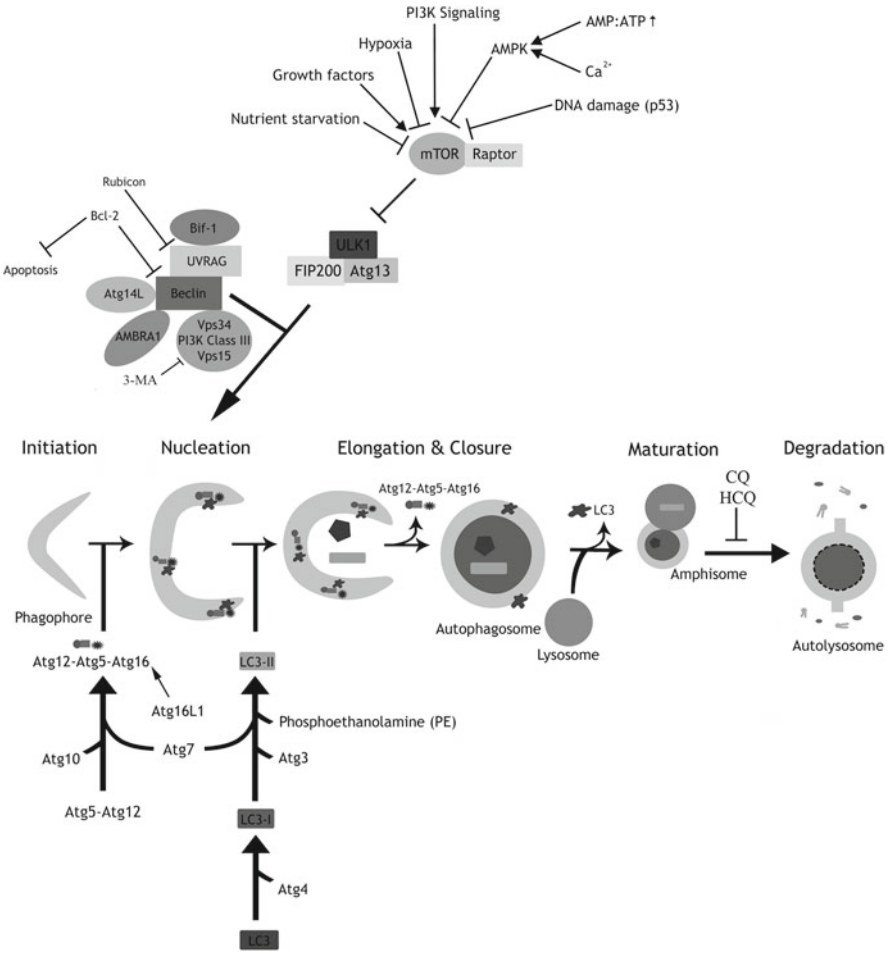

Fig. 8.2 Overview of the complexes involved in autophagosome formation. At least four important functional groups of autophagy-related gene proteins are required for autophagy: ULK1 protein-kinase complex and vacuolar sorting protein 34–Beclin 1 class III phosphoinositide 3-kinase (PI3K) complex regulate autophagy initiation; the Atg9-Atg2-Atg18 complex regulates the expansion of the phagophore assembly site; and the Atg5-Atg12-Atg16 and LC3 conjugation systems regulate the elongation of autophagosome membranes. Phosphatidylethanolamine (*PE*)-conjugated LC3 (called *LC3-II*) remains on the isolation membranes and autophagosome membranes, whereas the Atg12-Atg5-Atg16 complex transiently associates with the isolation membranes and dissociates from the autophagosome membranes. Pharmacological inhibitors of the autophagy process are 3-methyladenine, which inhibits PI3K, and autophagosome formation, while chloroquine (*CQ*) and hydroxychloroquine (*HCQ*) block autophagosome maturation by increasing the pH of the lysosomes

the proteasome and facilitates autophagic degradation of ubiquitinated protein aggregates. It is known to activate the nuclear factor erythroid 2-related factor 2 (NRF2) (Inami et al. 2011). This transcription factor turns on the antioxidant gene transcription that allows cells to protect themselves from oxidative stress.

8.1.2 Maturation of the Autophagosome Through the Endocytic Pathway

Autophagosomes are subsequently transformed to an amphisome after fusion with an endosome/lysosome. During this step, endocytosis and autophagy share machinery for the maturation of the autophagosome. A functional endocytic pathway from the early endosomes to the late endosomes and including multivesicular bodies is essential to maintaining an efficient autophagic flux. Several proteins, including members of the Rab GTPase family, Vps, and endosomal sorting complexes required for transport, have been identified as regulating each step of this process and are described in recent reviews (Lamb et al. 2013). Rab7 is an important element that controls endosomal maturation and lysosome traffic, and its activity is regulated in part by its GTPase-activating proteins and by the PI3K complex formed by Rubicon-UVRAG-Rab7 (Liang et al. 2008; Sun et al. 2010). Rab7 activity is inhibited by its binding with Rubicon and UVRAG (Liang et al. 2008). However, when the level of Rab7 increases until a threshold point, binding with Rubicon is lost and UVRAG can activate the HOPS (homotypic fusion and Vps) complex, which further increases Rab7 activity, promoting fusion with lysosomes (Zlatic et al. 2011; Peralta et al. 2010).

8.1.3 The End of the Road Through the Lysosome

Lysosomes have emerged as an important platform of mTORC1 signaling and regulation. It has been shown that lysosomal genes are regulated by the transcription factor EB (TFEB), which also controls the major steps of the autophagy pathway (autophagosome formation, autophagosome fusion with lysosomes, and degradation of cargo) linking autophagy to lysosomal biogenesis (Sardiello et al. 2009; Settembre et al. 2011). Under stress or aberrant lysosomal storage conditions, TFEB translocates from the cytoplasm to the nucleus and induces lysosomal biogenesis (Settembre et al. 2012). Other groups demonstrated that the lysosomal reformation that occurs during autophagy is regulated by mTORC1 and that TFEB phosphorylation and nuclear translocation are coordinately regulated by mTORC1 (Yu et al. 2010; Pena-Llopis et al. 2011). At the peak of autophagy, lysosomes are consumed by their fusion with autophagosomes, but after a prolonged period of autophagy, mTORC1 is reactivated (inhibits autophagy) and induces lysosomal biogenesis through TFEB activation (Yu et al. 2010).

The mTORC1 pathway that regulates cell growth in response to numerous cues, including amino acids, has been found on the lysosomal surface, its site of activation (Pena-Llopis et al. 2011; Korolchuk et al. 2011). Although the mechanism that elucidates every step of this process is not completely understood, elegant studies indicate that Rag GTPases (a heterodimeric complex of RagA/B and RagC/D GTPases), also located on the lysosomes, and vacuolar-type H+-ATPase (V-ATPase)

form a signaling system that is necessary for amino acid sensing by mTORC1 (Bar-Peled et al. 2012; Zoncu et al. 2011; Settembre et al. 2012; Sancak et al. 2010). Under nutrient-rich conditions, mTOR is located on peripheral lysosomes, where it becomes activated and promotes cell growth and inhibits autophagy, whereas mTOR and lysosomes are clustered in the perinuclear area during starvation, leading to induction of autophagy. This location facilitates the fusion of autophagosomes with lysosomes and autophagosome synthesis (by inhibiting mTOR activity) (Korolchuk and Rubinsztein 2011). The lysosome distribution depends, in part, on their being transported along microtubules, a process mediated by Arl8 (a small GTPase) and KIF2 (a kinesis family member) (Korolchuk et al. 2011). pHi has been shown to affect lysosome positioning, where acidification redistributes lysosomes from their predominantly perinuclear location toward the cell periphery and correlates with increased mTOR activity and inhibition of autophagy (Korolchuk et al. 2011; Heuser 1989).

8.2 Role of Autophagy in Cancer

Cells with defects in autophagy accumulate misfolded proteins, ubiquitinated aggregates, lipid droplets, and damaged organelles (mostly mitochondria, peroxisomes, and endoplasmic reticulum) that could lead to accumulation of reactive oxygen species (ROS), metabolic stress, and toxicity. Disruption of autophagy has been associated with cancer. The consequences of autophagy defects in cancer are complex, and new advances indicate that it could be linked to the tumor stages (White 2012; Mah and Ryan 2012; Janku et al. 2011). Autophagy can suppress tumors by preventing accumulation of toxic waste and tumor initiation, but it can also help cancer cells survive under metabolic stress and promote tumors once the tumor is established. Understanding the role of autophagy in cancer is critical because inhibition or activation of autophagy can be therapeutically applicable to killing cancer cells.

8.2.1 Autophagy in Tumor Suppression and Tumor Initiation

Genetic deletion of Beclin-1 is among the first evidence that autophagy can prevent tumor formation: mice with allelic loss of Beclin-1 are partially defective for autophagy and have increased spontaneous malignancies (Qu et al. 2003; Yue et al. 2003). Similarly, humans with Beclin-1 deletion have a higher frequency of leukemia, lymphomas, and tumors of the liver, lung, breast, ovarian, and prostate (Liang et al. 1999; Aita et al. 1999). Further studies of knockout mice demonstrated that basal autophagy is essential for viability because deletion of both Beclin-1 alleles induces embryonic lethality. In addition, the activation of Beclin-1 inhibits cell proliferation in vitro and tumor growth. Moreover, mice deficient in Atg4C develop fibrosarcomas (Marino et al. 2007), whereas a loss of Atg5 and Atg7 improve the risk of benign liver tumors (Takamura et al. 2011).

It has been shown that autophagy activation can prevent necrotic cell death in apoptosis-deficient cells, a process that may cause local inflammation and promote tumor growth (White et al. 2010). One explanation for the role of autophagy in tumor suppression has been linked to its ability to removed toxic waste during the initiation stage of tumorigenesis. Cells with deregulation in autophagy cause impaired mitochondria and accumulation of ROS, which promote genotoxic stress through DNA damage (Mathew et al. 2007; Degenhardt et al. 2006). This could lead to the loss of mitochondrial potential membrane, activation of phosphatase and tensin homolog–induced putative linase-1 (*PINK1*) and induction of *PARK2*, an E3 ubiquitin ligase involved in mitophagy (Arena et al. 2013). *PARK2* is a tumor suppressor gene, and mutations of it have been observed in glioblastomas and colon and lung cancers (Veeriah et al. 2010; Poulogiannis et al. 2010).

Another possibility by which autophagy may prevent cancer is through p62 (Mathew et al. 2009). In unstressed cells, NRF2 activity is inhibited by its binding to kelch-like ECH-associated protein 1 (*KEAP1*), which inactivates the antioxidant defense genes and stimulates proteasomal degradation (Copple et al. 2010; Lau et al. 2010). In autophagy-defective cells or in the presence of oxidative stress, *KEAP1* is modified and its binding with NRF2 is lost (Lau et al. 2010). Then, *p62* can bind and sequester *KEAP1*, promoting NRF2 activation, antioxidant defense, and survival. Therefore, autophagy is necessary to prevent p62 accumulation and NRF2 activation that could promote tumorigenesis.

8.2.2 Autophagy in Tumor Progression

Autophagy is induced as an alternative source of energy and metabolites to maintain cell survival during nutrient starvation or metabolic or other stress such as hypoxia, ischemia, and proteasome inhibition. Almost all of these conditions are observed in established tumors. Under stress conditions, autophagy protects dormant cells from damage (White 2012). When the conditions are more favorable or return to normal, these cells can recover and grow. Then, autophagy can provide a survival advantage to tumor cells, allowing them to adapt to metabolic stress found in the tumor microenvironment; a variety of mechanisms have been proposed to support this. It has been shown that the Bcl-2/adenovirus E1B interacting protein (*BNIP3*), a downstream target of hypoxia-inducible factor (HIF)-1α, can induce autophagy by disrupting the Beclin-1–Bcl-2 complex to release Beclin-1 in response to a hypoxic microenvironment (Bellot et al. 2009). Amino acid and glucose deprivation found in the tumor microenvironment have been correlated with a higher level of autophagosomes and deletion of essential Atgs, which induces tumor cell death associated with the hypoxic regions. Recent studies indicate that human cancer tissues with a low level of Beclin-1 have been associated with worse prognosis in esophageal (Chen et al. 2009), colon (Li et al. 2009), and pancreatic cancer (Kim et al. 2011). In addition, tumors from Beclin-1-deficient mice are more aggressive under hypoxic conditions, a mechanism that could be regulated through the HIF-2α

(Lee et al. 2011). Other studies reported that autophagy is triggered to protect cancer cells from nutrient deprivation by activation of AMP-activated protein kinase (AMPK), a sensor of energy status. AMPK activation limits translation initiation and protein synthesis through the inhibition of elongation factor 2 (EF2) and the inhibition of mTOR, leading to the induction of autophagy (Horbinski et al. 2010).

By studying the role of autophagy in cancer, several groups have noticed that cancer cells have a high level of basal autophagy, even in unstressed conditions. White and colleagues showed that activated cells expressing Ras are dependent on autophagy to survive starvation, and biallelic deletion of Atg5 or Atg7 decrease tumor growth of RAS-transformed epithelial cells in the kidneys of nude mice (Guo et al. 2011). This study indicated that autophagy is required to maintain functional mitochondrial and oxidative metabolism necessary to Ras-expressing tumor growth. Autophagy can also promote metastasis and cell survival in response to microenvironmental stresses (Kenific et al. 2010). High expression of *LC3* and *Beclin-1* are correlated with poor survival and a shorter disease-free period in pancreatic and nasopharyngeal carcinomas, respectively (Fujii et al. 2008; Wan et al. 2010). It is interesting to note that γ-aminobutyric acid type A receptor-associated protein (*GABARAP*), a member of the *LC3* family, is a new prognostic marker for colorectal carcinoma because its overexpression is associated with reduced survival (Miao et al. 2010).

8.3 Autophagy and Cell Death as Targets for Anticancer Therapy

There are a number of molecules targeting various proteins of the apoptosis pathway. Some groups of these molecules – such as ABT-263 (www.clinicaltrials.gov identifier NCT00743028), AT-101 (NCT00275431), and GX15-070MS or Obatoclax (NCT00600964) – affect the activation or balance of the Bcl-2 protein family, tipping the scale toward apoptosis, while others block the inhibitor apoptosis proteins, including AT-406 (NCT01078649), ENZ-3042 (NCT01186328), HGS-1029 (NCT00708006), and LCL-161 (NCT01098838), thus inducing the apoptosis pathway. On the other hand, elucidation of the molecular mechanisms involved in autophagy indicates crosstalk between the apoptotic and autophagic pathways (Amelio et al. 2011; Ouyang et al. 2012). For example, inhibition of apoptosis can induce autophagy, whereas inhibition of autophagy can stimulate apoptosis (Maiuri et al. 2007). In addition, both pathways can be activated through similar proteins, among them, the complex formed by Beclin-1 and Bcl-2 (Kang et al. 2011). Depending on the anticancer agents and the cell type, drugs can have a lethal effect in response to autophagy induction through the influence of the anti-apoptotic effect of Bcl-2 or the phosphorylation of Jun kinase (Wei et al. 2008). Among other proteins that could be involved in the crosstalk between apoptosis and autophagy are the activation of *p53*, which transcriptionally increases the signaling of AMPK; death-associated protein kinase (DAPK1); tuberous sclerosis protein 2 (TSC2); and ULK1/2 (Feng 2010). Autophagy may also protect against tumorigenesis by

Table 8.2 Clinical trials of monotherapeutic agents that induce autophagy

Agents	Target	Condition	Clinical trial
Autophagosome formation			
Imatinimb	Bcr-Abl	Leukemia	NCT00079313
Temsirolimus	mTOR	Renal cell carcinoma	NCT00494091
Everolimus	mTOR	Renal cell carcinoma	NCT00422344
Amiodarone	mTOR	Atrial fibrillation	NCT00845780
Sunitinib	VEGFR	Renal cell carcinoma	NCT01441661
AZD8055	mTOR	Solid tumors	NCT00973076
Sorafenib	VEGFR	Renal cell carcinoma	NCT00478114
Arsenic trioxide	BNIP3	Liver	NCT00582400
Perifosine	Akt	Prostate cancer	NCT00058214
Metformin	AMPK	Ovarian cancer	NCT01208740
Autophagosome maturation			
STF-62247	Unknown	Renal cell carcinoma	
CQ	Lysosomotropic agent	Small-cell lung cancer Ductal carcinoma	NCT01575782 NCT01023477
HCQ	Lysosomotropic agent	Renal cell carcinoma	NCT01144169

Data are taken from www.ClinicalTrials.gov. *Akt* protein kinase B; *AMPK* AMP-activated protein kinase; *BNIP3* BCL2/adenovirus E1B 19-kDa interacting protein 3; *CQ* chloroquine; *HCQ* hydroxychloroquine, *mTOR* mammalian target of rapamycin; *VEGFR* vascular endothelial growth factor receptor

limiting necrosis and chronic inflammation in response to metabolic stress, which is associated with the release of the proinflammatory HMGB1 (Degenhardt et al. 2006). A hypoxic tumor microenvironment, nutrient or amino acid levels, as well as the signaling pathway can influence the final outcome between cell death and survival when autophagy is induced. Whether cells can die from autophagy (autophagic cell death) or as a consequence of autophagy induction needs to be addressed.

8.3.1 Autophagy to Induce Cell Death

Various chemotherapeutic agents have been shown to induce autophagy and participate in the induction of cell death. Therefore, inhibition of autophagy using small interfering RNA targeting Atg5, Atg7, or Beclin-1 reduces death, suggesting that autophagy can eliminate tumor cells (Amaravadi et al. 2011; Janku et al. 2011). Table 8.2 summarize agents that have been reported to have anticancer effects as monotherapies. One of the most targeted approaches to killing cancer cells in response to autophagy is through the mTOR pathway. This process regulates cell proliferation and protein translation, and its inhibition induces autophagy as well as cell cycle arrest and apoptosis. The strong induction of autophagy in vivo in response to everolimus, a chemotherapeutic agent targeting mTOR, reduces the growth of advanced pancreatic tumors (Yao et al. 2010) and leukemia (Crazzolara et al. 2009).

Table 8.3 Clinical trials of combined agents modulating autophagy

Molecule name	Condition	Clinical stage	Clinical trials identifier
HCQ-docetaxel	Prostate cancer	Phase II	NCT00786682
HCQ-gemcitabine	Pancreatic cancer	Phase I	NCT01506973
HCQ-MK2206	Advanced solid tumors and prostate and kidney cancers	Phase I	NCT01480154
HCQ-everolimus	Renal cell carcinoma	Phase I	NCT01510119
HCQ-rapamycin	Relapsed or refractory myeloma	Phase I	NCT01689987
HCQ-erlotinib	Lung cancer	Phase II	NCT01026844
HCQ-sirolimus or vorinostat	Advanced solid cancers	Phase I	NCT01266057 NCT01023737
HCQ-temozolomide	Advanced solid tumors	Phase I	
HCQ-sunitinib	Advanced solid tumors	Phase I	NCT00813423
HCQ-bortezomib	Multiple myeloma	Phase I/II	NCT00568880
Rapamycin-sunitinib	Advanced non-small-cell lung cancer	Phase I	NCT00555256
Everolimus-BEZ235	Advanced solid tumors, metastatic breast cancer, and metastatic renal cell carcinoma	Phase I	NCT01482156
Rapamycin-trastuzumab	Metastatic breast cancer	Phase II	NCT00411788

Data are taken from (www.ClinicalTrials.gov)

Furthermore, temsirolimus and everolimus have been approved for the treatment of renal cell carcinoma (RCC). In addition, radiation as well as many chemotherapeutic agents inducing DNA damage and *p53* activation have demonstrated a synergic effect in combination with everolimus to kill cancer cells (O'Reilly et al. 2011). Other drugs inhibiting Bcl-2 and activating Beclin-1 in apoptosis-defective cells show a potential effect on cell killing by the formation of autophagosomes. Obatoclax is a Bcl-2 inhibitor that induces cell death. However, when apoptosis is functional, Obatoclax could promote both autophagy and apoptosis to kill acute lymphoblastic leukemia and non-small-lung cancer (Heidari et al. 2010; McCoy et al. 2010).

8.3.2 Inhibition of Autophagy to Improve Anticancer Treatments

As an alternative, autophagy could be associated with chemoresistance by protecting the survival of cancer cells. Thus, inhibition of the autophagic flux synergized the killing effect of chemotherapeutic agents in many tumor types. The mechanism by which autophagy inhibition increases cell death could be associated with a switch toward other types of cell death, such as apoptosis, necrosis, or necroptosis. Chloroquine (CQ) and its analog hydroxychloroquine (HCQ) are antimalarial agents that increase the pH of the lysosome and then inhibit the fusion between autophagosome and lysosome (Amaravadi et al. 2011) (Table 8.3). For example, administration of the Akt inhibitor MK2206 in combination with HCQ is in clinical

trials of pancreatic, kidney, and many advanced tumors. HCQ with everolimus is in a phase I clinical trial of RCC. The combination of HCQ, radiation, and temozolomide are in clinical trials of patients with glioblastomas (www.ClinicalTrials.gov identifier NCT00486603). In chronic myelogenous leukemia, cell death is observed by the combined treatment with CQ and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (Carew et al. 2007). Finally, HCQ has been shown to potentiate the anticancer effect of 5-fluorouracil in colon cancer (Sasaki et al. 2010). Two other autophagy inhibitors have recently been identified in preclinical trials. The first inhibitor is lucanthone, or Myricil D, an existing drug that is used for the treatment of schistosomal parasites (Clarkson and Erasmus 1984). While earlier investigations have shown that lucanthone inhibits topoisomerase 2 activity, a more recent study defined a novel mechanism of action for lucanthone that includes the disruption of lysosomal function, inhibition of autophagy, and induction of apoptosis (Carew et al. 2011). In breast carcinoma cell lines, lucanthone is tenfold more potent that CQ and shows a better safety profile than CQ or HCQ. The second autophagy inhibitor is Lys05. This new drug accumulates more easily within the lysosome, increasing pH more effectively compared to HCQ (McAfee et al. 2012). Similar to lucanthone, Lys05 displayed significantly higher anticancer activity than CQ or HCQ in preclinical models, without inducing significant observable toxicity. These two new autophagy inhibitors need to be further investigated as potential therapeutic anticancer agents.

8.4 Synthetic Lethality and Autophagy in Anticancer Drug Discovery

8.4.1 Synthetic Lethality in the Context of Cancer

Advances in cell and molecular biology have improved our knowledge of the mechanism by which cells escape death to become cancerous. The expansion of "omics" technology, from genomic through metabolomic, have identified specific mutations of genes or altered RNA and protein signaling that are responsible for different types of cancer. As discussed earlier, targeted therapy is an active area of research that has expanded the type and modality of treatments (alone or in combination). It is unfortunate that few of them show clinical efficacy, but the ones that received approval from the US Food and Drug Administration have improved survival of inflexible cancers, including RCC (Motzer et al. 2006, 2007, 2008; Gu et al. 2005; Hudes et al. 2007; Escudier et al. 2007a, b), pancreatic cancers (Moore et al. 2007), and non-small-cell lung cancers (Ansari et al. 2009; Shepherd et al. 2005). One promising approach to develop targeted therapy against tumor cells and spare normal tissue is based on synthetic lethality, which targets specific mutations in cancer genes that are not altered in normal cells (Chan and Giaccia 2011). Synthetic lethality is the genetic interaction of two genes, both of which are involved in essential processes (Hartman

et al. 2001). When either gene is mutated alone, the cell remains viable. However, the combination of these two mutations induces cell death (Hartman et al. 2001; Kaelin 2005; Hartwell et al. 1997). Chemical or RNA interference screens have made it possible to search for synthetic lethal interactions in mammalian cells (Farmer et al. 2005; Jiang et al. 2009). Thus, deregulation of an oncogene or inactivation of a tumor suppressor gene can be specifically targeted through synthetic lethality to kill tumor cells. This approach could be advantageous and facilitate the development of treatment with a single agent because only cancer cells with the specific mutation will die. The normal cells will not be affected by the therapy, and side effects from chemotherapy will be reduced. Synthetic lethality could also be used in combination with drugs and/or radiation or in patients with relapsed cancer, providing the opportunity to use lower doses of cytotoxic drugs, improve the therapeutic index of cytotoxic drugs, and reduce off-target effects. Driving mutation in cancer cells can change at different stages of tumor development – from the primary tumor to metastases – and therefore synthetic lethality could be useful to target the epithelial-to-mesenchymal transition as well as metastatic disease for which there are few options of effective treatment.

The first example of synthetic lethal interaction in cancer cells came from the mutation affecting the gene *BRCA1/2* and the enzyme poly (ADP ribose) polymerase (PARP). The tumor suppressor protein BRCA is an important player in the reparation of double-strand DNA breaks, and mutations affecting these genes have been reported in breast and ovarian cancers (Hall et al. 1992; Casey et al. 1993; Parikh and Advani 1996). In addition, PARP is an important protein that repairs single-stand DNA breaks (Petermann et al. 2005). By using pharmacological inhibitors or small interfering/small hairpin RNA targeting PARP in *BRCA*-mutated cells, studies indicate that these cells were not able to repair double-strand DNA breaks and recombination lesions and that they die by apoptosis (Bryant et al. 2005; Farmer et al. 2005). The identification of the lethal interaction between *BRCA* mutations and PARP inhibitors has been investigated in cancer cells, and several PARP inhibitors are currently in clinical trials (phase I/II/III) for the treatment of breast and ovarian cancer with the inactivated *BRCA1/2* gene (Tutt et al. 2010; Fong et al. 2009; Hutchinson 2010). These studies demonstrated proof of the concept that synthetic lethality can be useful in (and are possible for) targeting cancer cells. Some researchers and pharmaceutical companies are working to develop this killing approach in association with other oncogenes that are frequently disrupted in cancer, such as the oncogenes *Ras* and *Myc* (Chan and Giaccia 2011). New drugs (triphenyltetrazolium and a sulphinylcytidine derivative) (Torrance et al. 2001), the inhibitor apoptosis protein survivin (Sarthy et al. 2007), and cyclin-dependent kinase 4 (Puyol et al. 2010) have been identified by independent screening and demonstrate some potential as *KRAS* inhibitors. Otherwise, other large screens performed in *Ras*-mutated cells and pathways governing the mitotic machinery or the proteasomes showed synthetic lethal interaction with *Ras* (Scholl et al. 2009; Luo et al. 2009). Among other examples of synthetic lethal interaction, inhibition of aurora kinase B or death receptor 5 agonists induced killing in cells overexpressing *Myc* (Wang et al. 2004; Yang et al. 2010).

8.4.2 Synthetic Lethality and Autophagy in RCC

RCC, the most common form of kidney cancer, is particularly challenging because it is resistant to standard cytotoxic therapies. The overall 5-year survival rate ranges from 85 % in patients with local tumors treated by partial or total nephrectomy to 10 % in patients with advanced or metastatic RCC (Motzer et al. 1996). There is no curative treatment for RCC, and these patients are diagnosed at an advanced stage because no symptoms are associated with kidney tumors until they are quite large. Current targeted therapies used to treat RCC (e.g., bevacizumab, sunitinib) have focused on anti-angiogenic agents targeting vascular endothelial growth factor and its receptor and agents that inhibit mTOR (e.g., temsirolimus, everolimus). Although these agents demonstrate efficiency in RCC, the clinical response to these therapies is generally short-lived, suggesting that tumor growth might be supported by alternative sources of nutrients, such as autophagy (Patel et al. 2006).

Biallelic inactivation of the von Hippel-Lindau (*VHL*) tumor suppressor gene arises in up to 85 % of RCC cases. Mutation and/or hypermethylation, which inactivate the *VHL* gene, are also responsible for the hereditary VHL cancer syndrome that affects 1 in 36,000 individuals (Maher 2004). These patients inherit a faulty allele of *VHL* and are predisposed to the development of renal cysts, RCC, retinal and central nervous system hemangioblastomas, and pheochromocytomas (Maher 2004; Kaelin 2008). Tumor development is caused by somatic inactivation of the remaining wild-type allele (Young et al. 2009; Nickerson et al. 2008; Patard et al. 2009). Because VHL is a common and early event in the development of RCC, targeting its inactivation represents a promising target for the development of new therapies. High-throughput screening using a small interfering RNA library or small molecules have been performed in *VHL*-deficient RCC in two independent studies. The first approach used a library of small hairpin RNA against about 100 different kinases and distinguished CDK6, hepatocyte growth factor receptor (also known as MET), and mitogen-activated protein kinase 1 (MAP2K1), which have the ability to reduce growth of *VHL*-inactivated cells (Bommi-Reddy et al. 2008). A recent study reported that microRNA-1826 reduced the expression of β-catenin and *MAP2K1* in RCC and inhibits the proliferation of *VHL*-deficient cells by inducing G_1 arrest and apoptosis (Hirata et al. 2012).

The second approach used a library of 64,000 small molecules to find drugs that specifically kill RCC lacking *VHL* without affecting the viability of the cells with the functional *VHL* gene (Turcotte et al. 2008). This study identified two classes of compounds: ST-31 inhibited the survival of *VHL*-deficient cells through GLUT1 and HIF-1α (Chan et al. 2011), whereas STF-62247 killed *VHL*-mutated cells by inducing autophagy (Turcotte et al. 2008). Moreover, they showed that reducing levels of Atg5, Atg7, and Atg9 rescued the survival of *VHL*-deficient cells in response to STF-62247, indicating that autophagy induction is required for cell death. Turcotte et al. recently investigated the autophagy machinery and found that the in vitro and in vivo sensitivity of *VHL*-deficient RCC in response to STF-62247 is associated with a default in the autophagic process involving lysosomal

degradation, which ultimately leads to cell death. In accordance with this, cells lacking *VHL* expression accumulate autophagic vacuoles that are not degraded by lysosomes, thus interfering with the clearance of damaged organelles and misfolded or aggregated proteins in response to STF-62247. Furthermore, lysosomes in these cells undergo labialization or lysosome permeabilization, which also contributes to cell death. Production of ROS that are not detoxified by the cells, lysosomotropic agents, microtubule-stabilizing agents, protein kinase C, phospholipase A_2, and lipids are the mechanisms speculated to induce lysosome permeabilization (Kreuzaler and Watson 2012).

8.5 Conclusion and Future Directions

The field of cancer research has made significant progress in recent years. New techniques have identified genetic alterations associated with different types of cancer. In parallel, advances in drug screening using small interfering RNA libraries and/or small molecules have expanded drug design and the development of targeted therapies. Using these approaches, new anticancer agents or novel uses of existing drugs are in clinical trials or have been approved for treatment. Exciting drugs exploiting synthetic lethality have gained attention as a new type of anticancer therapy. Searching for synthetic lethal interaction between two genes or drug-gene interactions represent a promising approach to kill tumor cells and leave normal cells healthy. Cancer cells evade programmed cell death to initiate tumor formation, and research has identified nonapoptotic mechanisms for how cells survive or die in response to a drug. The role of autophagy in cancer is complex: it can help prevent tumor initiation, overcome resistance to anticancer therapy, promote cytoprotection in established tumors, and may help to eradicate malignant cells. Inhibitors of the autophagic flux, including CQ and HCQ, used alone or in combination with chemotherapeutic agents and/or radiation, are currently in clinical trials of several types of cancer. In addition, drugs that induce autophagy and provoke cell death show encouraging results. Overall, other screens using synthetic lethality and our knowledge of cell death mechanisms could open a new field of oncology, helping to design monotherapy agents or a combination of cytotoxic chemotherapy and radiation.

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