Current Cancer Research

Jin-Ming Yang *Editor*

Targeting Autophagy in Cancer Therapy

Springer

Current Cancer Research

Series Editor
Wafik El-Deiry

More information about this series at http://www.springer.com/series/7892

Jin-Ming Yang
Editor

Targeting Autophagy in Cancer Therapy

Springer

Editor
Jin-Ming Yang
Department of Pharmacology
The Pennsylvania State University
College of Medicine
Hershey, PA, USA

ISSN 2199-2584 ISSN 2199-2592 (electronic)
Current Cancer Research
ISBN 978-3-319-42738-6 ISBN 978-3-319-42740-9 (eBook)
DOI 10.1007/978-3-319-42740-9

Library of Congress Control Number: 2016949552

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

Preface

Over the past decade or so, an ever-increasing body of scientific evidence points to the functional role and unmistakable importance of autophagy in cancer. But can autophagy be successfully exploited as a target in effective cancer therapy? It is now widely believed that modulating the activity of autophagy through targeting regulatory components in the autophagy machinery may impact the development, progression, and therapeutic outcome of cancer. Therefore, autophagy has been considered a novel and promising target for drug discovery/development and therapeutic intervention for cancer; in fact, targeting of autophagy as a therapeutic strategy in cancer has already been explored in-depth and has shown great promise. The purpose of this volume is to provide the latest updates on the current status and a unique perspective on autophagy-based cancer therapy. This volume in the Springer series, *Current Cancer Research,* will cover a wide range of topics, including an overview of autophagy as a therapeutic target in cancer, autophagy modulators as cancer therapeutic agents, implications of micro RNA-regulated autophagy in cancer therapy, modulation of autophagy through targeting PI3 kinase in cancer therapy, targeting autophagy in cancer stem cells, and the roles of autophagy in cancer immunotherapy. In addition, this volume presents a chapter on the application of system biology and bioinformatics approaches to discovering cancer therapeutic targets in the autophagy regulatory network. This comprehensive volume is intended to be useful to a wide range of basic and clinical scientists, including cancer biologists, autophagy researchers, pharmacologists, and clinical oncologists who wish to delve more deeply into this exciting new research area.

Although there are already several excellent books that cover the biology and molecular biology of autophagy and their association with cancer development and progression, this is the first book devoted solely to dealing with targeting autophagy in cancer therapy. As the implications and importance of autophagy in cancer therapy have been increasingly appreciated, this timely and unique volume assembled by leading scientists in this field should prove its usefulness and value in understanding, exploring, developing, and promoting autophagy-based cancer therapy. This volume has the following distinguishing features: (1) it is the first book solely focusing on autophagy as a target in cancer therapy; (2) it is a comprehensive

discussion on the roles of autophagy in currently available cancer treatments; (3) it is a timely complement to the book (volume 8): *Autophagy and Cancer*, 2013, in this series. Finally, I want to sincerely thank all of the authors for their contribution. It is my earnest hope that this volume will serve as a catalyst for further exploration and investigation of autophagy-based cancer therapy.

Hershey, PA, USA Jin-Ming Yang

Contents

Chapter 1
Autophagy as a Therapeutic Target in Cancer

Jenny Mae Samson and Andrew Thorburn

Abstract Autophagy is the process by which cellular material is delivered to the lysosome for degradation and recycling. Macroautophagy involves delivery of macromolecules and organelles to double membrane vesicles called autophagosomes that fuse with lysosomes leading to degradation of the contents of the autophagosomes. Chaperone-mediated autophagy involves direct recognition of specific proteins by chaperone complexes that then directly deliver the protein target to the lysosome. Microautophagy involves direct lysosomal capture of cytoplasmic material. Of these three types, macroautophagy is by far the most studied and is known to have multiple roles in cancer development, progression and response to therapy. This has led to autophagy being widely viewed as a potential therapeutic target in cancer. Important questions that must be answered include: Which tumors should or should not be treated by direct autophagy inhibition? And, what is the best way to target autophagy for cancer therapy? In this overview we summarize the background and some current ideas about the answers to such questions.

Keywords Autophagy • Apoptosis • Cancer therapy • ATG7 • BRAF • KRAS

Autophagy is the process through which proteins, organelles, and other cellular contents are degraded in lysosomes. Macroautophagy involves the formation of double membrane vesicles called autophagosomes that engulf and sequester cellular material. The autophagosomes then fuse with lysosomes, generating autophagolysosomes, in which the lysosomal hydrolases degrade the delivered material into their macromolecular precursors for reuse. While the process of autophagy was first described in the early 1960s, it is only in the past 10–15 years that its role in cellular homeostasis (Kaur and Debnath 2015), as well as in many diseases (Kroemer 2015; Rubinsztein et al. 2012) has been recognized. Two other types of autophagy that do

J.M. Samson • A. Thorburn (✉)
Department of Pharmacology, University of Colorado School of Medicine,
Aurora, CO 80045, USA
e-mail: Andrew.Thorburn@ucdenver.edu

J.-M. Yang (ed.), *Targeting Autophagy in Cancer Therapy*,
Current Cancer Research, DOI 10.1007/978-3-319-42740-9_1

not involve autophagosomes have been characterized: chaperone-mediated autophagy and microautophagy. Chaperone-mediated autophagy (CMA) involves the direct recognition of proteins by heat shock protein hsc70 through an exposed amino acid (KFERQ) motif and subsequent delivery of the bound pair to the lysosome through the lysosomal protein LAMP2A (Arias and Cuervo 2011; Kaushik et al. 2011). Microautophagy is less well understood than either CMA or macroautophagy and may involve components of the autophagic machinery and endocytic pathways that allow direct engulfment of cytoplasmic material into the lysosome (Sahu et al. 2011). Most of the work related to autophagy in the context of cancer refers to macroautophagy, though recent work has demonstrated the importance of CMA in tumor growth and progression. Hereafter we use the term "autophagy" to mean macroautophagy.

As we will discuss, autophagy's involvement in cancer is confusing and oftentimes contradictory with both pro- and anti-tumor effects found in different contexts (Hippert et al. 2006; White 2012; Galluzzi et al. 2015) and during cancer therapy (Thorburn et al. 2014). In January 2016 a search of the ClinicalTrials.gov website with the search term "autophagy" returned 60 clinical studies across the world. The majority of these clinical studies deliberately attempt to inhibit autophagy during cancer therapy usually together other anti-cancer treatments. The first cancer clinical trials of autophagy inhibitors were reported in 2014 (Barnard et al. 2014; Rangwala et al. 2014a, b; Rosenfeld et al. 2014; Vogl et al. 2014; Wolpin et al. 2014). These attempts to target autophagy in cancer therapy contrasts with only a few examples where deliberate autophagy manipulation is being attempted to treat other diseases (Kroemer 2015). Thus, despite the fact that arguments can be made for and against inhibiting autophagy in cancer and for the utility of autophagy manipulation in infectious disease, neurodegenerative disease, metabolic disease and many others (Kroemer 2015), it is in cancer treatment where we are furthest along in trying to apply these ideas in a clinical setting. It is also important to note that many current anti-cancer treatments themselves induce autophagy (Shen et al. 2011; Levy and Thorburn 2011). Conversely, some microtubule-targeting drugs such as paclitaxel inhibit autophagy (Veldhoen et al. 2013). This means that we are routinely affecting autophagy in cancer patients through their course of treatment whether we intend to or not. In this chapter, we focus on the deliberate targeting of autophagy and provide an overview of arguments for and against the direct manipulation of autophagy in cancer therapy.

Autophagy is regulated by a large set of evolutionarily conserved genes called *ATG* genes (Mizushima et al. 2011). The ATG proteins represent a variety of types of molecules including lipid and protein kinases and protein conjugating enzymes and scaffolding proteins many of which may represent novel drug targets. Indeed selective inhibitors of a lipid kinase, VPS34, (Bago et al. 2014; Dowdle et al. 2014; Ronan et al. 2014) and the protein kinase ULK1 (Egan et al. 2015; Petherick et al. 2015) were recently shown to inhibit autophagy and to have anti-tumor effects. One important source of confusion in the literature comes from the fact that all known autophagy regulators (i.e. ATG proteins) have other cellular roles as well (Subramani and Malhotra 2013). For example, loss of ATG7 inhibits autophagy, but ATG7 also

regulates p53 via autophagy-independent mechanisms (Lee et al. 2012). So, if *Atg7* deletion in a mouse model of cancer alters tumor growth (Guo et al. 2013a; Karsli-Uzunbas et al. 2014; Strohecker et al. 2013; Xie et al. 2015; Rosenfeldt et al. 2013), is this due to autophagy being inhibited or could it be due to an effect on p53? Similar examples arise with other essential autophagy regulators—e.g. ATG12 regulates apoptosis (Radoshevich et al. 2010; Rubinstein et al. 2011), ATG5 controls MAP kinases (Martinez-Lopez et al. 2013) and mitotic catastrophe (Maskey et al. 2013), while BECN1 controls cytokinesis (Thoresen et al. 2010). These effects are all autophagy-independent and could also affect tumor cell growth/survival. Without a known molecule that only regulates autophagy without affecting other biological activities, current best practice for *in vitro* experiments is to target multiple autophagy regulators and ensure that they all have similar effects on the phenotype being studied before concluding that autophagy affects that phenotype (Thorburn 2008, 2011). Such experimental rigor is more difficult *in vivo* but is, if anything, even more important if we are to avoid misinterpretation of experimental results. For example, it was believed that autophagy is critical for tuberculosis infection based on studies where mice lacking ATG5 were very susceptible to infection. However, more extensive studies targeting multiple ATGs in mice demonstrated that this susceptibility is not due to ATG5's role in autophagy but rather a unique function that is not seen when other ATGs are targeted (Kimmey et al. 2015).

Autophagy is often described as a mostly a non-selective process whereby any cellular material in the vicinity of the forming autophagosomes can be sequestered and eventually degraded. This idea is mistaken and oversimplified, as there are several types of selective autophagy. In particular, there are specific autophagic mechanisms for the degradation of mitochondria (mitophagy), intracellular bacteria (xenophagy) (Randow and Youle 2014), the endoplasmic reticulum and contents of the nucleus (Mochida et al. 2015), lipid droplets (lipophagy) (Singh et al. 2009), and damaged lysosomes (Maejima et al. 2013). These specific forms of autophagy potentially have important effects on tumors; for example, defective mitophagy has been shown to promote breast cancer metastasis (Chourasia et al. 2015). Specific proteins are also targeted for autophagic degradation, such as under conditions of iron depletion, where specific targeting of ferritin to autophagosomes takes place to allow release of iron (Mancias et al. 2014). Even in conditions where one might think that non-selective autophagy would be favored, e.g. amino acid starvation where autophagic degradation of any proteins would, at least in principle, provide amino acids to the cell, autophagy is highly selective such that some proteins are degraded while others are protected (Mathew et al. 2014). Thus, although we currently have a poor understanding of how cells determine which autophagy cargos are degraded under different circumstances, it seems likely that autophagy is largely—if not entirely—selective. This specificity in cargo delivery to autophagosomes is critical in understanding the biological effects of autophagy. For instance, it can explain how autophagy can promote apoptosis for one apoptosis inducer but not another (Gump et al. 2014; Thorburn 2014). Although understanding selective autophagy may be vital to effectively target autophagy therapeutically, at present we have no way to selectively affect cargo-specific autophagy. All the current clinical

trials mentioned above use lysosome-targeted pharmacological agents to target autophagy, namely chloroquine (CQ) or its derivative hydroxychloroquine (HCQ), which both inhibit the lysosome. An important caveat to bear in mind is that CQ can chemosensitize to other anti-cancer drugs through autophagy-independent mechanisms as well as by inhibiting autophagy (Maycotte et al. 2012; Eng et al. 2016), adding another layer to the complexity underlying the debate.

Autophagy is induced by diverse stresses such as nutrient deprivation, hypoxia, metabolic stress and many others and in most cases the induction of autophagy serves to protect cells from the insult. Thus, if cells are starved of amino acids they rapidly induce autophagy and, if that autophagy induction is prevented using either pharmacological inhibitors or genetic interference of the ATG genes that regulate autophagy, many more cells die as a result of the amino acid starvation. Such experiments clearly show that autophagy is protective in this context. Moreover, because such effects are seen in response to a wide variety of pro-apoptotic stimuli, autophagy is widely thought to protect against apoptosis. This protective effect is generally the basis for the idea that autophagy inhibition will chemosensitize tumor cells to other drugs that underlies the numerous clinical trials mentioned above (Thorburn et al. 2014). Contrarily, early papers that considered autophagy's roles in the cancer chemotherapy response (e.g. to the anti-estrogen tamoxifen (Bursch et al. 1996), or in apoptosis-deficient cells treated with DNA damaging drugs (Shimizu et al. 2004)), often concluded that the induction of autophagy by the therapeutic agent caused tumor cell death. One of the first clear demonstrations that autophagy can protect against chemotherapy came from studies in a Myc-driven lymphoma model (Amaravadi et al. 2007). More recently, many studies with diverse anti-cancer drugs including DNA damaging agents and other traditional cytotoxics as well as newer "targeted" agents have tended to conclude that autophagy is primarily protective against cancer therapy (Thorburn et al. 2014). In fact, it is clear that both in response to physiological signals (e.g. during development) and exogenous pro-death stimuli, autophagy can both promote and inhibit cell death/apoptosis (Fitzwalter and Thorburn 2015).

As mentioned above, in the 60-odd ongoing clinical trials identified using the search term "autophagy," the majority are attempting to inhibit autophagy with HCQ. The basis for these studies is twofold. First, an idea that inhibition of autophagy will, by itself, inhibit tumor growth. Second the idea that autophagy inhibition will make another anti-cancer treatment more effective. Let's next consider the rationales for both ideas.

1.1 Inhibiting Autophagy on Its Own for Anti-cancer Treatment

Why think that autophagy inhibition could have an anti-tumor effect even in the absence of other treatments? This concept is based on a large body of data showing that direct interference with autophagy (e.g. by knocking down or knocking out *ATG* genes) can, by itself, inhibit tumor growth and/or promote tumor cell death

(Guo et al. 2013b). The first such demonstration from Jay Debnath's group showed that autophagy was important for transformation by KRAS (Lock et al. 2011) and many of the other studies identifying tumors that require autophagy have also tended to focus on tumors with RAS pathway mutations. In fact, a series of studies in genetically engineered mouse models from Eileen White and colleagues (e.g. Guo et al. 2011, 2013a; Karsli-Uzunbas et al. 2014; Strohecker et al. 2013; Xie et al. 2015), Alec Kimmelman (Yang et al. 2011, 2014), Kevin Ryan (Rosenfeldt et al. 2013), and Josef Penninger (Rao et al. 2014) all focused on tumors driven by mutant KRAS or BRAF and demonstrated anti-tumor effects upon genetic inhibition of autophagy by knock out of an essential ATG. Recent studies in flies also showed autophagy-dependence of RAS-driven tissue overgrowth, however, when tissue growth was driven by the Notch pathway, autophagy had the opposite effect (Pérez et al. 2015). This study is important because it establishes that autophagy's roles in controlling tissue growth can be different in different contexts. An important role for BRAF mutation was demonstrated in pediatric brain tumors where brain tumor cells with wild-type BRAF demonstrated no dependency on autophagy (Levy and Thorburn 2012) (i.e. autophagy inhibition had little effect on tumor cell growth) whereas similar tumor cells that harbored BRAF mutations displayed a high degree of autophagy-dependence such that genetic or pharmacological inhibition of autophagy was sufficient to kill them (Levy et al. 2014).

In some of the mouse studies, autophagy inhibition switched the tumor from an adenoma or adenocarcinoma to a less aggressive tumor type called an oncocytoma (Guo et al. 2013a; Strohecker et al. 2013). In humans, oncocytomas are known to display defects in autophagy (Joshi et al. 2015). The majority of the tumor studies listed above involved activation of an oncogene at the same time and in the same cells that autophagy was inhibited by tissue-specific knockout of an essential ATG; consequently in these cases tumor development and progression all took place without the ability of the tumor cells to perform canonical autophagy. This observation begs the question, what happens if a tumor is allowed to form first, then autophagy is inhibited? Such studies are important because they mimic what a therapeutic intervention might look like (if we had a perfectly effective inhibitor of autophagy that worked as well as knockout of an essential ATG). In one study (Karsli-Uzunbas et al. 2014) such an experiment was done. This work showed that although complete, inducible knockout of ATG7 in adult mice is eventually toxic (the mice die of infection or eventual neurodegeneration consistent with known functions of autophagy that protect organisms), when autophagy was inhibited in the whole animal, this blocked the growth and promoted regression, as well as switching to more benign oncocytomas of pre-existing KRAS mutant lung tumors. An important concern raised by this study is that because all the mice eventually died of neurodegeneration and others were more susceptible to bacterial infection, we must be cautious about autophagy inhibition as a therapeutic strategy. In humans we could presumably never achieve as efficient and irreversible an inhibition of autophagy as we get with the complete knockout of a gene in a mouse so such concerns may be alleviated given two points: first, with pharmacological autophagy inhibitors that would be used in people we could stop treatment to allow recovery from side effects, and second, we would be unlikely to have as complete inhibition of the process.

These studies have led to the suggestion that KRAS mutant or BRAF mutant tumors are the best candidates for autophagy inhibition therapy (Mancias and Kimmelman 2011; Thorburn and Morgan 2015). However some studies have shown that KRAS mutation does not always lead to tumor cells being more sensitive to autophagy inhibition. In an aforementioned mouse study described above, it was demonstrated that p53 status switched autophagy from being tumor promoting in KRAS-driven pancreas cancer to being tumor inhibiting. Therefore, when KRAS-driven pancreas tumors developed with germline loss of p53, autophagy inhibition caused increase growth of the tumors while the same genetic manipulations demonstrated an anti-tumor effect of autophagy inhibition in p53 wildtype mice (Rosenfeldt et al. 2013). It is important to note that germline loss of p53 is not the way that p53 is inactivated during human pancreas cancer development, and that another study where p53 loss occurred in a manner more analogous to what occurs during human pancreas cancer found that p53 status did not alter the beneficial effect of autophagy inhibition (Yang et al. 2014). The explanation for these differences is unknown but imply an important role for p53 function during the development of a tumor in determining whether autophagy promotes or inhibits tumor growth. Other evidence suggests that RAS mutation by itself does not predict whether a tumor cell will be inhibited or increased in its growth when autophagy is blocked. In genetically defined human tumor cells where normal cells are immortalized then transformed by sequential introduction of telomerase, inhibition of p53 and RB then transformed with oncogenic HRAS, some cells showed that transformation was associated with increased dependence on autophagy (i.e. autophagy inhibition reduced growth) whereas other cells transformed in exactly the same stepwise fashion showed increased growth when autophagy was inhibited (Morgan et al. 2014). More recent analysis of a large number of RAS-mutant cell lines also concluded that growth of RAS mutant cell lines was not necessarily inhibited when autophagy was blocked (Eng et al. 2016). A recent study of pancreas tumors demonstrated a critical role for autophagy that was linked not to RAS mutation per se (which nevertheless occurs in the vast majority of pancreas tumors), but instead to increased activity of transcription factors that drive autophagy and allow efficient tumor cell metabolism that is necessary for sustaining cancer growth (Perera et al. 2015).

Although many studies have focused on RAS pathway driven tumors, an anti-tumor effect of genetic inhibition of autophagy is also seen in murine tumors driven by different oncogenic drivers (Huo et al. 2013; Wei et al. 2011, 2014). This raises the question of whether some tumor cells are indeed highly dependent on autophagy but that this dependency can occur with or without RAS mutation. A study in breast cells (Maycotte et al. 2014) where over 100 different autophagy regulators were targeted with pooled shRNAs attempted to circumvent the problem noted above whereby non-autophagy functions of ATG genes confound conclusions of autophagy being important for a biological effect. This is important because all the studies described above where autophagy was targeted and shown to be critical for tumor growth came to this conclusion after inhibiting only one or two ATGs.

The Maycotte et.al. study (Maycotte et al. 2014) found that some breast cancer cell lines were highly dependent on autophagy for growth in the absence of added

stress such as amino acid starvation. These cells tended to lose shRNAs that target positive regulators of autophagy. In other words, when autophagy was inhibited the cells had a selective disadvantage for continued growth. Other breast cancer cell lines could be grown for weeks with no apparent selection against shRNAs that target autophagy suggesting that these cells don't care about autophagy unless they are stressed (e.g. by amino acid starvation). Importantly, only tumors grown from autophagy-dependent tumor cells displayed any inhibition of growth *in vivo* when autophagy was inhibited with CQ. These effects were associated with changes in STAT3 signaling such that in autophagy-dependent breast cancer cells STAT3 signaling and cell growth required autophagy, while in autophagy-independent breast cancer cells STAT3 activity was not controlled by autophagy. In a follow-up paper (Maycotte et al. 2015), it was shown that autophagy-dependent cells require autophagy to promote secretion of the cytokine IL6, which is critical for promoting tumor cell growth and cancer stem cell activity. In contrast, autophagy-independent cells demonstrated no decrease in IL6 secretion when autophagy was inhibited, instead secreting more IL6 when autophagy was inhibited. These effects were also associated with markedly different changes in gene expression patterns upon autophagy inhibition between autophagy-dependent and autophagy-independent tumor cells. Another study showed that autophagy-dependent secretion of IL6 and, most likely of other signaling molecules, is critical for breast cancer cell invasion and metastasis (Lock et al. 2014). Although we have a very poor understanding of the full nature of the differences between autophagy-dependent and autophagy-independent cancer cells, these experiments suggest that the central differences of behavior in response to targeting autophagy reveal themselves because autophagy controls completely different and sometimes opposing pathways in different cancer cells. These studies indicate that in some tumor cells (i.e. the ones that are highly dependent on autophagy) continued tumor growth, survival and perhaps invasion all depend on autophagy, making a strong argument for autophagy inhibition as a therapeutic approach in cancer. However, it is imperative to understand that this only occurs in *some* tumor cells. In others, not only might autophagy inhibition be ineffective, it may be counterproductive and actually increase tumor growth. It will be critical to dissect the biology that underlies these differences if we are to know which tumors to target and which not to target with autophagy inhibitors.

1.2 Inhibiting Autophagy to Make Other Treatments More Effective

The majority of the clinical trials where autophagy is deliberately targeted involve an autophagy inhibitor used in combination with another drug. A large amount of literature describes chemosensitization effects of autophagy inhibition (Levy and Thorburn 2011; Maycotte and Thorburn 2011; Thorburn et al. 2014; Rebecca and Amaravadi 2015). Some of these effects may be due to the other anti-cancer drug itself increasing autophagy. For example, mTOR inhibitors are potent inducers of

autophagy and it can be shown that co-ordinate inhibition of autophagy can sensitize to mTOR inhibitors (Xie et al. 2013). The interpretation of such studies is that the autophagy induced by the drug reduces its ability to kill the cancer cells, so that the addition of an autophagy inhibitor (such as CQ) blocks this protection thus sensitizing to the other drug. This finding has led to clinical studies of such combinations (Rangwala et al. 2014a). However, as with the findings of opposing effects when autophagy is targeted on its own in different contexts, recent work suggests that the even the same combination of drugs in autophagy-dependent and -independent tumors can show different effects. Thus, in the autophagy-dependent and autophagy-independent breast cell lines described in the previous section (Maycotte et al. 2014), the same drug combination (doxorubicin plus the autophagy inhibitor chloroquine) was only synergistic in autophagy-dependent breast cancer cells and was sometimes actually antagonistic in autophagy-independent breast cancer cells. Similar results were found in autophagy-dependent BRAF mutant versus autophagy-independent BRAF wild-type brain cancer cells (Levy et al. 2014). There are also cases where specific anti-cancer drugs have been reported to require autophagy in order to elicit their anti-tumor effect. Epidermal Growth Factor Receptor (EGFR) signaling was reported to inhibit autophagy by phosphorylating and disrupting the activity of the autophagy regulator Beclin 1 (BECN1) (Wei et al. 2013). Moreover, EGFR inhibitors, which are commonly used to treat EGFR mutant tumors, were found to restore this autophagy activity. The resultant anti-tumor effect was found to require autophagy restoration, implying that in this case, adding on an autophagy inhibitor would prevent the EGFR inhibitor from working. Such studies suggest that choosing the correct drug to combine with autophagy inhibitors will be important and, possibly even more critical, will be selecting such a combination for the appropriate tumor cells.

The aforementioned examples are attempting to increase the efficacy of a drug that has at least some activity. One of the major problems in cancer therapy comes when tumors acquire resistance to a drug, which may develop in myriad ways (Holohan et al. 2013), including the increased expression of drug efflux pumps and the reduced ability of the tumor cell to undergo apoptosis. For targeted therapies such as kinase inhibitors that block specific signaling pathways, resistance commonly arises due to activation of the same pathway downstream of the inhibited kinase or activation of a parallel signaling pathway. In some cases we are starting to obtain evidence that autophagy inhibition can be used as a way to circumvent such acquired resistance. The best examples to date come from studies in BRAF mutant tumors. It has been shown that autophagy inhibitors can synergize with BRAF inhibitors (Goodall et al. 2014). However, autophagy inhibitors may also be able to do more: they can also overcome resistance to the BRAF inhibitor. In BRAF mutant melanoma, the acquisition of resistance in the clinic to the RAF inhibitor vemurafenib was shown to correlate with higher numbers of autophagosomes, suggesting that increased autophagy occurs as the tumors evolve to become resistant against the BRAF inhibitor and undergo more endoplasmic reticulum stress (Ma et al. 2014). Moreover, *in vitro* experimental selection for vemurafenib resistance could be reversed in this situation via autophagy inhibition. We also have at least one case

where such an effect—adding an autophagy inhibitor reverses resistance to the BRAF inhibitor—may be true in a patient. In this case (Levy et al. 2014), a patient with a BRAF mutant brain tumor was treated for almost a year with vemurafenib but then had a recurrence indicating that her tumor had acquired resistance to the drug. The patient was then treated with a combination of vemurafenib and CQ, which caused tumor regression. Importantly, this particular patient was taken off the BRAF inhibitor for periods of time while continuing treatment with CQ and this caused the tumor to start growing again. Thus, in this patient, neither BRAF inhibitor alone nor the autophagy inhibitor alone was effective at inhibiting tumor growth and causing regression; only the combination works. These data are consistent with the idea that it is the reversal of resistance that is the key benefit of autophagy inhibition. This patient also demonstrates that autophagy inhibition therapy can be done for extensive periods of time (in this case more than 2 years as of the time of writing) without signs of toxicity due to the autophagy inhibitor. Therefore, the concerns raised with the mice where inducible knockout of the *Atg7* gene led to death caused by neurodegeneration within a few months (Karsli-Uzunbas et al. 2014) may be less significant in practice when we are incompletely inhibiting autophagy in the clinic.

1.3 Potential Reasons Not to Inhibit Autophagy in Cancer Therapy

The previous discussion argues that autophagy inhibition may be worthwhile as an anti-cancer therapy alone or together with other drugs but only in some already existing tumors. Other studies raise different issues that have been used to argue against autophagy inhibition therapy. One possible reason is that autophagy may serve to suppress the development of new cancers. The rationale for this argument rests on the observation that several autophagy genes function as tumor suppressors when they knocked out in mice. For instance, BECN1 homozygous deletion leads to early embryonic lethality but heterozygous deletion causes increased incidence of cancer (Qu et al. 2003; Yue et al. 2003), suggesting that BECN1 is a haploinsufficient tumor suppressor. In human tumors, this interpretation has been challenged and it has been suggested that the apparent loss of BECN1 in human cancers is primarily due to loss of an adjacent gene, BRCA1 (Laddha et al. 2014). However, other studies suggest that such a bystander effect is not in play and that BECN1 is functioning as a tumor suppressor in some human breast cancers (Tang et al. 2015). In mice, mosaic deletion of ATG5 or liver-specific deletion of ATG7 leads to the development of benign liver adenomas that do not progress to aggressive cancer (Takamura et al. 2011). Deletion of other autophagy regulators in mice can also cause spontaneous cancer development. Examples include BIF-1 (Takahashi et al. 2007), ATG4C (Marino et al. 2007) and UVRAG (Liang et al. 2006), although this last example could be due to autophagy-independent functions in maintaining chromosome stability (Zhao et al. 2012). Studies similar to these have led to the

suggestion that autophagy may suppress the development of cancer even when it can promote cancer progression. In this case, one might expect that general inhibition of autophagy would cause pre-neoplastic lesions to progress faster.

The above examples consider the effect of autophagy in cancer to be primarily an autonomous effect on the behavior of the tumor cell itself; that is, autophagy may promote or inhibit growth of the cancer cell, cause it to be more or less likely to die, or affect the cell's ability to migrate and invade other tissues. Autophagy manipulation in one cell may also alter the way that neighboring cells behave. This could have repercussions for cancer development, progression, and response to therapy. The best example here concerns how dying tumor cells do or do not affect and engage the immune system. It was demonstrated that chemotherapy-induced immunogenic cell death of cancer cells requires that autophagy be functional in the dying tumor cells (Michaud et al. 2011). This effect was necessary for effective treatment of the tumor in immune competent mice but not in immune deficient animals demonstrating that the difference was due to how the immune system recognized the dying cancer cells rather than an effect on the efficiency of tumor cell killing by the chemotherapeutic itself. A mechanism was traced to a requirement for autophagy in the release of ATP from the dying cells. In other circumstances autophagy may be important in controlling the release of other immune stimulators such as the Damage Associated Molecular Pattern (DAMP) molecule HMGB1 (Thorburn et al. 2009). Autophagy may also be important in tumor antigen presentation (Li et al. 2012). Together, these findings would tend to suggest that autophagy inhibition during cancer therapy should reduce immunogenic tumor cell killing, i.e. arguing against trying to target autophagy. However, even here the situation is complicated. It has been shown that autophagy inhibition with CQ significantly enhances T cell-mediated tumor killing after Interleukin 2 immunotherapy (Liang et al. 2012). Hypoxia-induced autophagy impairs natural killer (NK) cell-mediated killing of tumor cells and autophagy inhibition was shown to enhance tumor elimination by NK cells *in vivo* (Baginska et al. 2013). Thus, as with the other competing effects discussed above, the benefits and caveats of targeting autophagy are also manifested when it comes to immunogenic tumor cell killing. These studies further emphasize how crucial it will be to understand the full spectrum of effects—both good and bad—that occur when autophagy is targeted during cancer therapy.

1.4 Conclusions

What should be clear from the above discussion (which is by no means definitive, many other studies arguing both for and against autophagy as a therapeutic target in cancer could have been discussed) is that there is no straightforward conclusion as to how, or even whether, we should try to target autophagy as a therapeutic approach to cancer. Numerous important questions remain to be answered and there is evidence both for and against the idea of targeting autophagy that we need to make sense of. Moreover, it is unclear how we should go about targeting autophagy.

Current clinical approaches focus on targeting the lysosome with drugs like HCQ, and other, more potent lysosomal inhibitors are also being developed (Goodall et al. 2014; Mcafee et al. 2012). The ability of lysosome inhibitors to chemosensitize through autophagy-independent mechanisms may also be useful (Maycotte et al. 2012; Eng et al. 2016). Earlier steps in the autophagy pathway can also be therapeutically targeted (Bago et al. 2014; Dowdle et al. 2014; Ronan et al. 2014; Egan et al. 2015; Petherick et al. 2015). Will these be better than lysosome-targeted drugs for cancer therapy? Perhaps the most critical issue is to determine which tumors should be targeted and which should not. This is a pressing issue because accumulating evidence suggests that not only might targeting autophagy be ineffective in some tumors, in those tumors that are not highly dependent on autophagy, it may be counterproductive to do so. If we try to inhibit autophagy in the wrong tumor, this may not only fail to slow tumor growth, it might enhance growth. Targeting autophagy in the wrong tumor may not only fail to make another drug more efficacious, it might make that drug less effective. Added complexity comes when one considers how altering autophagy in cancer cells may affect how other cells (e.g. immune cells) recognize the tumor cells. It will require a much more sophisticated understanding of how these effects work and how their balance determines the final outcome if we are to effectively pursue autophagy as a therapeutic target in cancer.

Given this complexity, one might propose not even to try targeting autophagy in cancer therapy. However, this is not an option; not only do we have ongoing clinical trials whose interpretation will require that we better understand this process and what it means for cancer cell behavior, we already know that even if we wanted to avoid targeting autophagy we couldn't do so. Most of our current anti-cancer treatments themselves affect autophagy, so we are routinely affecting autophagy during cancer therapy whether we like it or not. The way forward is to understand how the various competing effects of autophagy on cancer treatment and cancer/tumor behavior occur. Fortunately, the field is now poised to do so.

References

Amaravadi, R. K., Yu, D., Lum, J. J., Bui, T., Christophorou, M. A., Evan, G. I., et al. (2007). Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *Journal of Clinical Investigation, 117*, 326–336.

Arias, E., & Cuervo, A. M. (2011). Chaperone-mediated autophagy in protein quality control. *Current Opinion in Cell Biology, 23*, 184–189.

Baginska, J., Viry, E., Berchem, G., Poli, A., Noman, M. Z., Van Moer, K., et al. (2013). Granzyme B degradation by autophagy decreases tumor cell susceptibility to natural killer-mediated lysis under hypoxia. *Proceedings of the National Academy of Sciences of the United States of America,* 110, 17450–17455.

Bago, R., Malik, N., Munson, M. J., Prescott, A. R., Davies, P., Sommer, E., et al. (2014). Characterization of VPS34-IN1, a selective inhibitor of Vps34, reveals that the phosphatidylinositol 3-phosphate-binding SGK3 protein kinase is a downstream target of class III phosphoinositide 3-kinase. *The Biochemical Journal, 463*, 413–427.

Barnard, R. A., Wittenburg, L. A., Amaravadi, R. K., Gustafson, D. L., Thorburn, A., & Thamm, D. H. (2014). Phase I clinical trial and pharmacodynamic evaluation of combination hydroxy-

chloroquine and doxorubicin treatment in pet dogs treated for spontaneously occurring lymphoma. *Autophagy, 10*, 1415–1425.

Bursch, W., Ellinger, A., Kienzl, H., Torok, L., Pandey, S., Sikorska, M., et al. (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.

Chourasia, A. H., Tracy, K., Frankenberger, C., Boland, M. L., Sharifi, M. N., Drake, L. E., et al. (2015). Mitophagy defects arising from BNip3 loss promote mammary tumor progression to metastasis. *EMBO Reports, 16*, 1145–1163.

Dowdle, W. E., Nyfeler, B., Nagel, J., Elling, R. A., Liu, S., Triantafellow, E., et al. (2014). Selective VPS34 inhibitor blocks autophagy and uncovers a role for NCOA4 in ferritin degradation and iron homeostasis in vivo. *Nature Cell Biology, 16*(11), 1069–1079.

Egan, D. F., Chun, M. G. H., Vamos, M., Zou, H., Rong, J., Miller, C. J., et al. (2015). Small Molecule Inhibition of the autophagy kinase ULK1 and Identification of ULK1 substrates. *Molecular Cell, 59*, 285–297.

Eng, C. H., Wang, Z., Tkach, D., Toral-Barza, L., Ugwonali, S., Liu, S., et al. (2016). Macroautophagy is dispensable for growth of KRAS mutant tumors and chloroquine efficacy. *Proceedings of the National Academy of Sciences of the United States of America,* 113, 182–187.

Fitzwalter, B. E., & Thorburn, A. (2015). Recent insights into cell death and autophagy. *The FEBS Journal, 282*, 4279–4288.

Galluzzi, L., Pietrocola, F., Pedro Bravo-San, J. M., Amaravadi, R. K., Baehrecke, E. H., Cecconi, F., et al. (2015). Autophagy in malignant transformation and cancer progression. *The EMBO Journal, 34*(7), 856–880.

Goodall, M. L., Wang, T., Martin, K. R., Kortus, M. G., Kauffman, A. L., Trent, J. M., et al. (2014). Development of potent autophagy inhibitors that sensitize oncogenic BRAF V600E mutant melanoma tumor cells to vemurafenib. *Autophagy, 10*, 1120–1136.

Gump, J. M., Staskiewicz, L., Morgan, M. J., Bamberg, A., Riches, D. W. H., & Thorburn, A. (2014). Autophagy variation within a cell population determines cell fate through selective degradation of Fap-1. *Nature Cell Biology, 16*, 47–54.

Guo, J. Y., Chen, H.-Y., Mathew, R., Fan, J., Strohecker, A. M., Karsli-Uzunbas, G., et al. (2011). Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes and Development, 25*, 460–470.

Guo, J. Y., Karsli-Uzunbas, G., Mathew, R., Aisner, S. C., Kamphorst, J. J., Strohecker, A. M., et al. (2013a). Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. *Genes and Development, 27*, 1447–1461.

Guo, J. Y., Xia, B., & White, E. (2013b). Autophagy-mediated tumor promotion. *Cell, 155*, 1216–1219.

Hippert, M. M., O'Toole, P. S., & Thorburn, A. (2006). Autophagy in cancer: Good, bad, or both? *Cancer Research, 66*, 9349–9351.

Holohan, C., Van Schaeybroeck, S., Longley, D. B., & Johnston, P. G. (2013). Cancer drug resistance: An evolving paradigm. *Nature Reviews Cancer, 13*, 714–726.

Huo, Y., Cai, H., Teplova, I., Bowman-Colin, C., Chen, G., Price, S., et al. (2013). Autophagy opposes p53-mediated tumor barrier to facilitate tumorigenesis in a model of PALB2-associated hereditary breast cancer. *Cancer Discovery, 3*, 894–907.

Joshi, S., Tolkunov, D., Aviv, H., Hakimi, A. A., Yao, M., Hsieh, J. J., et al. (2015). The genomic landscape of renal oncocytoma identifies a metabolic barrier to tumorigenesis. *Cell Reports, 13*, 1895–1908.

Karsli-Uzunbas, G., Guo, J. Y., Price, S., Teng, X., Laddha, S. V., Khor, S., et al. (2014). Autophagy is required for glucose homeostasis and lung tumor maintenance. *Cancer Discovery, 4*, 914–927.

Kaur, J., & Debnath, J. (2015). Autophagy at the crossroads of catabolism and anabolism. *Nature Reviews Molecular Cell Biology, 16*, 461–472.

Kaushik, S., Bandyopadhyay, U., Sridhar, S., Kiffin, R., Martinez-Vicente, M., Kon, M., et al. (2011). Chaperone-mediated autophagy at a glance. *Journal of Cell Science, 124*, 495–499.

Kimmey, J. M., Huynh, J. P., Weiss, L. A., Park, S., Kambal, A., Debnath, J., et al. (2015). Unique role for ATG5 in neutrophil-mediated immunopathology during M. tuberculosis infection. *Nature, 528*, 565–569.

Kroemer, G. (2015). Autophagy: A druggable process that is deregulated in aging and human disease. *Journal of Clinical Investigation, 125*, 1–4.

Laddha, S. V., Ganesan, S., Chan, C. S., & White, E. (2014). Mutational landscape of the essential autophagy gene BECN1 in human cancers. *Molecular Cancer Research, 12*, 485–490.

Lee, I. H., Kawai, Y., Fergusson, M. M., Rovira, I. I., Bishop, A. J. R., Motoyama, N., et al. (2012). Atg7 modulates p53 activity to regulate cell cycle and survival during metabolic stress. *Science, 336*, 225–228.

Levy, J. M. M., Thompson, J. C., Griesinger, A. M., Amani, V., Donson, A. M., Birks, D. K., et al. (2014). Autophagy inhibition improves chemosensitivity in BRAFV600E brain tumors. *Cancer Discovery, 4*, 773–780.

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

Levy, J. M., & Thorburn, A. (2012). Modulation of pediatric brain tumor autophagy and chemosensitivity. *Journal of Neuro-Oncology, 106*, 281–290.

Li, Y., Hahn, T., Garrison, K., Cui, Z. H., Thorburn, A., Thorburn, J., et al. (2012). The vitamin E analogue alpha-TEA stimulates tumor autophagy and enhances antigen cross-presentation. *Cancer Research, 72*, 3535–3545.

Liang, X., de Vera, M. E., Buchser, W. J., Romo De Vivar Chavez, A., Loughran, P., Beer Stolz, D., et al. (2012). Inhibiting systemic autophagy during interleukin 2 immunotherapy promotes long-term tumor regression. *Cancer Research, 72*, 2791–2801.

Liang, C., Feng, P., Ku, B., Dotan, I., Canaani, D., Oh, B. H., et al. (2006). Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nature Cell Biology, 8*, 688–698.

Lock, R., Kenific, C. M., Leidal, A. M., Salas, E., & Debnath, J. (2014). Autophagy-dependent production of secreted factors facilitates oncogenic RAS-driven invasion. *Cancer Discovery, 4*, 466–479.

Lock, R., Roy, S., Kenific, C. M., Su, J. S., Salas, E., Ronen, S. M., et al. (2011). Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. *Molecular Biology of the Cell, 22*, 165–178.

Ma, X.-H., Piao, S.-F., Dey, S., McAfee, Q., Karakousis, G., Villanueva, J., et al. (2014). Targeting ER stress-induced autophagy overcomes BRAF inhibitor resistance in melanoma. *Journal of Clinical Investigation, 124*, 1406–1417.

Maejima, I., Takahashi, A., Omori, H., Kimura, T., Takabatake, Y., Saitoh, T., et al. (2013). Autophagy sequesters damaged lysosomes to control lysosomal biogenesis and kidney injury. *The EMBO Journal, 32*, 2336–2347.

Mancias, J. D., & Kimmelman, A. C. (2011). Targeting autophagy addiction in cancer. *Oncotarget, 2*, 1302–1306.

Mancias, J. D., Wang, X., Gygi, S. P., Harper, J. W., & Kimmelman, A. C. (2014). Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature, 509*, 105–109.

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

Martinez-Lopez, N., Athonvarangkul, D., Mishall, P., Sahu, S., & Singh, R. (2013). Autophagy proteins regulate ERK phosphorylation. *Nature Communications, 4*, 2799.

Maskey, D., Yousefi, S., Schmid, I., Zlobec, I., Perren, A., Friis, R., et al. (2013). ATG5 is induced by DNA-damaging agents and promotes mitotic catastrophe independent of autophagy. *Nature Communications, 4*, 2130.

Mathew, R., Khor, S., Hackett, S. R., Rabinowitz, J. D., Perlman, D. H., & White, E. (2014). Functional role of autophagy-mediated proteome remodeling in cell survival signaling and innate immunity. *Molecular Cell, 55*, 916–930.