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### Authors

Galluzzi, Lorenzo  
Pietrocola, Federico  
Bravo-San Pedro, José Manuel  
et al.

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## Review

# Autophagy in malignant transformation and cancer progression

Lorenzo Galluzzi<sup>1,2,3,4\*,†,‡</sup>, Federico Pietrocola<sup>1,2,3,†</sup>, José Manuel Bravo-San Pedro<sup>1,2,3,†</sup>, Ravi K Amaravadi<sup>5</sup>, Eric H Baehrecke<sup>6</sup>, Francesco Cecconi<sup>7,8</sup>, Patrice Codogno<sup>4,9,10,11</sup>, Jayanta Debnath<sup>12</sup>, David A Gewirtz<sup>13</sup>, Vassiliki Karantza<sup>14</sup>, Alec Kimmelman<sup>15</sup>, Sharad Kumar<sup>16</sup>, Beth Levine<sup>17,18,19</sup>, Maria Chiara Maiuri<sup>1,2,3</sup>, Seamus J Martin<sup>20</sup>, Josef Penninger<sup>21</sup>, Mauro Piacentini<sup>22,23</sup>, David C Rubinsztein<sup>24</sup>, Hans-Uwe Simon<sup>25</sup>, Anne Simonsen<sup>26</sup>, Andrew M Thorburn<sup>27</sup>, Guillermo Velasco<sup>28,29</sup>, Kevin M Ryan<sup>30,‡</sup> & Guido Kroemer<sup>1,2,4,31,32,‡</sup>

## Abstract

Autophagy plays a key role in the maintenance of cellular homeostasis. In healthy cells, such a homeostatic activity constitutes a robust barrier against malignant transformation. Accordingly, many oncoproteins inhibit, and several oncosuppressor proteins promote, autophagy. Moreover, autophagy is required for optimal anticancer immunosurveillance. In neoplastic cells, however, autophagic responses constitute a means to cope with intracellular and environmental stress, thus favoring tumor progression. This implies that at least in some cases, oncogenesis proceeds along

with a temporary inhibition of autophagy or a gain of molecular functions that antagonize its oncosuppressive activity. Here, we discuss the differential impact of autophagy on distinct phases of tumorigenesis and the implications of this concept for the use of autophagy modulators in cancer therapy.

**Keywords** adaptive stress responses; Beclin 1; inflammation; KRAS; mitophagy

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1 Equipe 11 labellisée pas la Ligue Nationale contre le Cancer, Centre de Recherche des Cordeliers, Paris, France

2 INSERM, U1138, Paris, France

3 Gustave Roussy Cancer Campus, Villejuif, France

4 Université Paris Descartes, Sorbonne Paris Cité, Paris, France

5 Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA

6 Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, MA, USA

7 Cell Stress and Survival Unit, Danish Cancer Society Research Center, Copenhagen, Denmark

8 IRCCS Fondazione Santa Lucia and Department of Biology, University of Rome Tor Vergata, Rome, Italy

9 Institut Necker Enfants-Malades (INEM), Paris, France

10 INSERM, U1151, Paris, France

11 CNRS, UMR8253, Paris, France

12 Department of Pathology and Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA, USA

13 Department of Pharmacology, Toxicology and Medicine, Virginia Commonwealth University, Richmond, Virginia, VA, USA

14 Merck Research Laboratories, Rahway, NJ, USA

15 Division of Genomic Stability and DNA Repair, Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

16 Centre for Cancer Biology, University of South Australia, Adelaide, SA, Australia

17 Center for Autophagy Research, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

18 Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX, USA

19 Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA

20 Department of Genetics, Trinity College, The Smurfit Institute, Dublin, Ireland

21 Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, Austria

22 Department of Biology, University of Rome Tor Vergata, Rome, Italy

23 National Institute for Infectious Diseases IRCCS 'Lazzaro Spallanzani', Rome, Italy

24 Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK

25 Institute of Pharmacology, University of Bern, Bern, Switzerland

26 Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

27 Department of Pharmacology, University of Colorado School of Medicine, Aurora, CO, USA

28 Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University of Madrid, Madrid, Spain

29 Instituto de Investigaciones Sanitarias San Carlos (IdiSSC), Madrid, Spain

30 Cancer Research UK Beatson Institute, Glasgow, UK

31 Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France

32 Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, France

\*Corresponding author. Tel: +33 1 44277661; E-mail: deadoc@vodafone.it

†These authors contributed equally to this work

‡These authors share senior co-authorship

## Introduction

Macroautophagy (herein referred to as autophagy) is a mechanism that mediates the sequestration of intracellular entities within double-membraned vesicles, so-called autophagosomes, and their delivery to lysosomes for bulk degradation (He & Klionsky, 2009). Autophagosomes derive from so-called phagophores, membranous structures also known as 'isolation membranes' whose precise origin remains a matter of debate (Lamb *et al*, 2013). Indeed, the plasma membrane, endoplasmic reticulum (ER), Golgi apparatus, ER-Golgi intermediate compartment (ERGIC), and mitochondria have all been indicated as possible sources for phagophores (Lamb *et al*, 2013). Upon closure, autophagosomes fuse with lysosomes, forming so-called autolysosomes, and their cargo is exposed to the catalytic activity of lysosomal hydrolases (Mizushima & Komatsu, 2011). The degradation products of the autophagosomal cargo, which includes sugars, nucleosides/nucleotides, amino acids and fatty acids, can be transported back to the cytoplasm and presumably re-enter cellular metabolism (Fig 1) (Rabinowitz & White, 2010; Galluzzi *et al*, 2013). Of note, the molecular machinery that mediates autophagy is evolutionary conserved, and several components thereof have initially been characterized in yeast (He & Klionsky, 2009).

In physiological scenarios, autophagy proceeds at basal levels, ensuring the continuous removal of superfluous, ectopic or damaged (and hence potentially dangerous) entities, including organelles and/or portions thereof (Green *et al*, 2011). Baseline autophagy mediates a key homeostatic function, constantly operating as an intracellular quality control system (Mizushima *et al*, 2008; Green *et al*, 2011). Moreover, the autophagic flux can be upregulated in response to a wide panel of stimuli, including (but not limited to) nutritional, metabolic, oxidative, pathogenic, genotoxic and proteotoxic cues (Kroemer *et al*, 2010). Often, stimulus-induced autophagy underlies and sustains an adaptive response to stress with cytoprotective functions (Kroemer *et al*, 2010; Mizushima & Komatsu, 2011). Indeed, the pharmacological or genetic inhibition of autophagy generally limits the ability of cells to cope with stress and restore homeostasis (Mizushima *et al*, 2008; Kroemer *et al*, 2010). This said, regulated instances of cell death that causally depend on the autophagic machinery have been described (Denton *et al*, 2009; Denton *et al*, 2012b; Liu *et al*, 2013b; Galluzzi *et al*, 2015). The detailed discussion of such forms of autophagic cell death, however, is beyond the scope of this review.

Autophagy is tightly regulated. The best characterized repressor of autophagic responses is mechanistic target of rapamycin (MTOR) complex I (MTORC1) (Laplante & Sabatini, 2012). Thus, several inducers of autophagy operate by triggering signal transduction cascades that result in the inhibition of MTORC1 (Inoki *et al*, 2012). Among other effects, this allows for the activation of several proteins that are crucial for the initiation of autophagic responses, such as unc-51-like autophagy-activating kinase 1 (ULK1, the mammalian ortholog of yeast Atg1) and autophagy-related 13 (ATG13) (Hosokawa *et al*, 2009; Nazio *et al*, 2013). A major inhibitor of MTORC1 is protein kinase, AMP-activated (PRKA, best known as AMPK), which is sensitive to declining ATP/AMP ratios (Mihaylova & Shaw, 2011). Besides inhibiting the catalytic activity of MTORC1, AMPK directly stimulates autophagy by phosphorylating ULK1 as well as phosphatidylinositol 3-kinase, catalytic subunit type 3 (PIK3C3, best known as VPS34) and Beclin 1 (BECN1, the

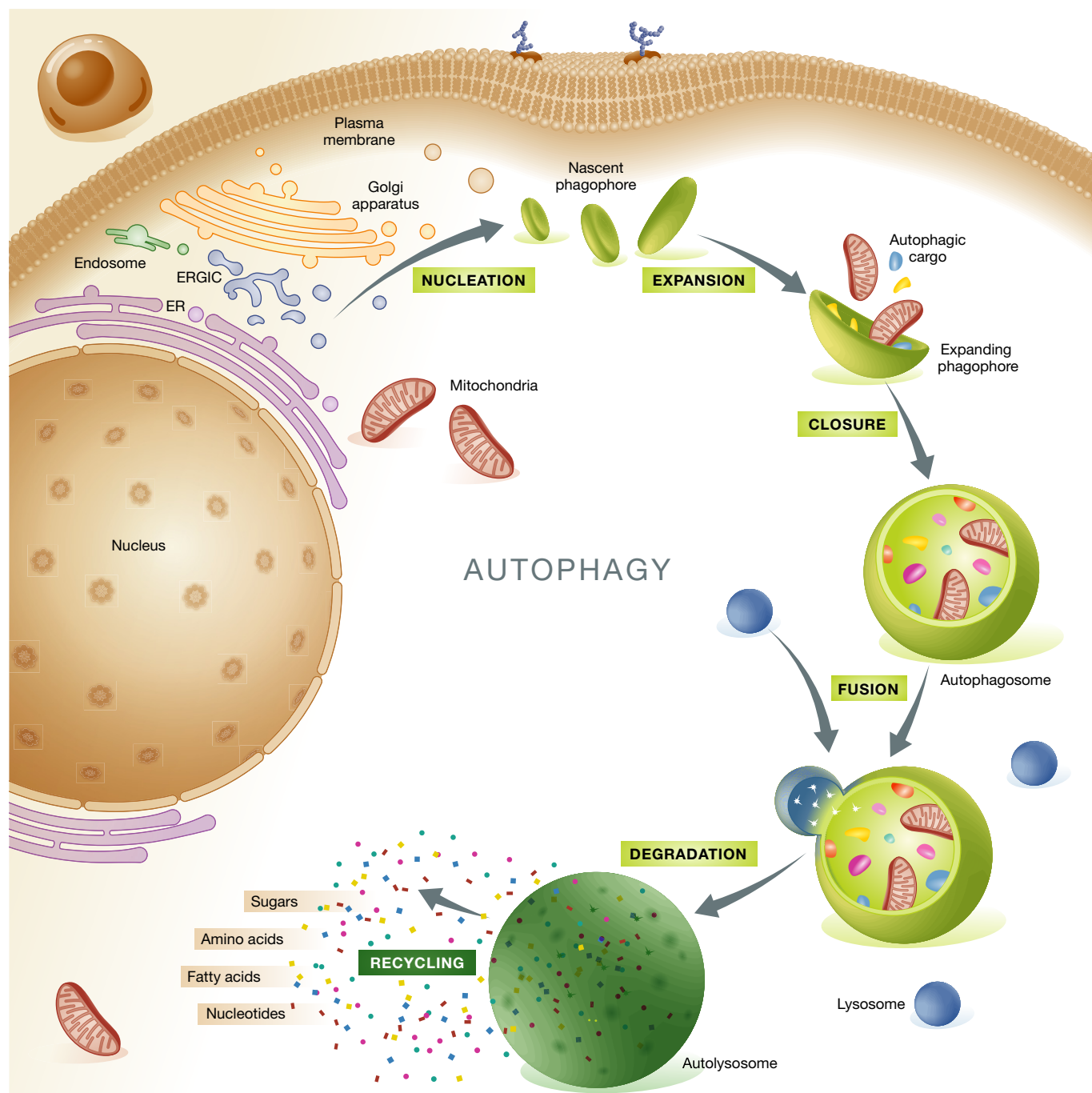
mammalian ortholog of yeast Atg6), two components of a multiprotein complex that produces a lipid that is essential for the biogenesis of autophagosomes, namely phosphatidylinositol 3-phosphate (Egan *et al*, 2011; Zhao & Klionsky, 2011; Kim *et al*, 2013). Autophagy also critically relies on two ubiquitin-like conjugation systems, both of which involve ATG7 (Mizushima, 2007). These systems catalyze the covalent linkage of ATG5 to ATG12 and ATG16-like 1 (ATG16L1), and that of phosphatidylethanolamine to proteins of the microtubule-associated protein 1 light chain 3 (MAP1LC3, best known as LC3) family, including MAP1LC3B (LC3B, the mammalian ortholog of yeast Atg8) (Mizushima, 2007). A detailed discussion of additional factors that are involved in the control and execution of autophagic responses can be found in Boya *et al* (2013).

Importantly, autophagosomes can either take up intracellular material in a relatively non-selective manner or deliver very specific portions of the cytoplasm to degradation, mainly depending on the initiating stimulus (Weidberg *et al*, 2011; Stolz *et al*, 2014). Thus, while non-selective forms of autophagy normally develop in response to cell-wide alterations, most often of a metabolic nature, highly targeted autophagic responses follow specific perturbations of intracellular homeostasis, such as the accumulation of permeabilized mitochondria (mitophagy), the formation of protein aggregates (aggrephagy), and pathogen invasion (xenophagy) (Okamoto, 2014; Randow & Youle, 2014). Several receptors participate in the selective recognition and recruitment of autophagosomal cargoes in the course of targeted autophagic responses (Rogov *et al*, 2014; Stolz *et al*, 2014). The autophagy receptor best characterized to date, that is, sequestosome 1 (SQSTM1, best known as p62), recruits ubiquitinated proteins to autophagosomes by virtue of an ubiquitin-associated (UBA) and a LC3-binding domain (Pankiv *et al*, 2007).

Owing to its key role in the preservation of intracellular homeostasis, autophagy constitutes a barrier against various degenerative processes that may affect healthy cells, including malignant transformation. Thus, autophagy mediates oncosuppressive effects. Accordingly, proteins with *bona fide* oncogenic potential inhibit autophagy, while many proteins that prevent malignant transformation stimulate autophagic responses (Morselli *et al*, 2011). Moreover, autophagy is involved in several aspects of anticancer immunosurveillance, that is, the process whereby the immune system constantly eliminates potentially tumorigenic cells before they establish malignant lesions (Ma *et al*, 2013). However, autophagy also sustains the survival and proliferation of neoplastic cells exposed to intracellular and environmental stress, hence supporting tumor growth, invasion and metastatic dissemination, at least in some settings (Kroemer *et al*, 2010; Guo *et al*, 2013b). Here, we discuss the molecular and cellular mechanisms accounting for the differential impact of autophagy on malignant transformation and tumor progression.

## Autophagy and malignant transformation

In various murine models, defects in the autophagic machinery caused by the whole-body or tissue-specific, heterozygous or homozygous knockout of essential autophagy genes accelerate oncogenesis. For instance, *Becn1*<sup>+/-</sup> mice (*Becn1*<sup>-/-</sup> animals are not viable) spontaneously develop various malignancies, including lymphomas as well as lung and liver carcinomas (Liang *et al*, 1999; Qu *et al*,



**Figure 1. General organization of autophagic responses.**

Autophagy initiates with the progressive segregation of cytoplasmic material by double-membraned structures commonly known as phagophores or isolation membranes. Phagophores nucleate from the endoplasmic reticulum (ER), but several other membranous organelles have been shown to contribute to their elongation, including the Golgi apparatus, ER-Golgi intermediate compartment (ERGIC), plasma membrane, mitochondria and recycling endosomes. Completely sealed phagophores, which are known as autophagosomes, fuse with lysosomes to form autolysosomes. This promotes the activation of lysosomal hydrolases and hence causes the breakdown of the autophagosomal cargo. The products of these catabolic reactions reach the cytosol via transporters of the lysosomal membrane and are recycled by anabolic or bioenergetic circuitries.

2003; Yue *et al*, 2003; Mortensen *et al*, 2011), and are more susceptible to parity-associated and *Wnt1*-driven mammary carcinogenesis than their wild-type counterparts (Cicchini *et al*, 2014). Similarly, mice lacking one copy of the gene coding for the BECN1 interactor

autophagy/beclin-1 regulator 1 (AMBRA1) also exhibit a higher rate of spontaneous tumorigenesis than their wild-type littermates (Cianfanelli *et al*, 2015). Mice bearing a systemic mosaic deletion of *Atg5* or a liver-specific knockout of *Atg7* spontaneously develop

benign hepatic neoplasms more frequently than their wild-type counterparts (Takamura *et al*, 2011). Moreover, carcinogen-induced fibrosarcomas appear at an accelerated pace in autophagy-deficient *Atg4c*<sup>-/-</sup> mice (Marino *et al*, 2007), as do *KRAS*<sup>G12D</sup>-driven and *BRAF*<sup>V600E</sup>-driven lung carcinomas in mice bearing lung-restricted *Atg5* or *Atg7* deletions, respectively (Strohecker *et al*, 2013; Rao *et al*, 2014). The pancreas-specific knockout of *Atg5* or *Atg7* also precipitates the emergence of *KRAS*<sup>G12D</sup>-driven pre-malignant pancreatic lesions (Rosenfeldt *et al*, 2013; Yang *et al*, 2014).

Several mechanisms can explain, at least in part, the oncosuppressive functions of autophagy. Proficient autophagic responses may suppress the accumulation of genetic and genomic defects that accompanies malignant transformation, through a variety of mechanisms. Reactive oxygen species (ROS) are highly genotoxic, and autophagy prevents their overproduction by removing dysfunctional mitochondria (Green *et al*, 2011; Takahashi *et al*, 2013) as well as redox-active aggregates of ubiquitinated proteins (Komatsu *et al*, 2007; Mathew *et al*, 2009). In addition, autophagic responses have been involved in the disposal of micronuclei arising upon perturbation of the cell cycle (Rello-Varona *et al*, 2012), in the degradation of retrotransposing RNAs (Guo *et al*, 2014), as well as in the control of the levels of *ras* homolog family member A (RHOA), a small GTPase involved in cytokinesis (Belaid *et al*, 2013). Finally, various components of the autophagic machinery appear to be required for cells to mount adequate responses to genotoxic stress (Karantzawa-Wadsworth *et al*, 2007; Mathew *et al*, 2007; Park *et al*, 2014). This said, the precise mechanisms underlying such genome-stabilizing effects remain elusive, implying that the impact of autophagy on DNA-damage responses may be indirect. Further investigation is required to shed light on this possibility.

Autophagy is intimately implicated in the maintenance of physiological metabolic homeostasis (Galluzzi *et al*, 2014; Kenific & Debnath, 2015). Malignant transformation generally occurs along with a shift from a predominantly catabolic consumption of glycolysis-derived pyruvate by oxidative phosphorylation to a metabolic pattern in which: (1) glucose uptake is significantly augmented to sustain anabolic reactions and antioxidant defenses, (2) mitochondrial respiration remains high to satisfy increased energy demands; and (3) several amino acids, including glutamine and serine, become essential as a means to cope with exacerbated metabolic functions (Hanahan & Weinberg, 2011; Galluzzi *et al*, 2013). Autophagy preserves optimal bioenergetic functions by ensuring the removal of dysfunctional mitochondria (Green *et al*, 2011), *de facto* counteracting the metabolic rewiring that accompanies malignant transformation. Moreover, the autophagic degradation of p62 participates in a feedback circuitry that regulates MTORC1 activation in response to nutrient availability (Linares *et al*, 2013; Valencia *et al*, 2014).

Autophagy appears to ensure the maintenance of normal stem cells. This is particularly relevant for hematological malignancies, which are normally characterized by changes in proliferation or differentiation potential that alter the delicate equilibrium between toti-, pluri- and unipotent precursors in the bone marrow (Greim *et al*, 2014). The ablation of *Atg7* in murine hematopoietic stem cells (HSCs) has been shown to disrupt tissue architecture, eventually resulting in the expansion of a population of bone marrow progenitor cells with neoplastic features (Mortensen *et al*, 2011). Along similar lines, the tissue-specific deletion of the gene coding

for the ULK1 interactor RB1-inducible coiled-coil 1 (RB1CC1, best known as FIP200) alters the fetal HSC compartment in mice, resulting in severe anemia and perinatal lethality (Liu *et al*, 2010). Interestingly, murine *Rb1cc1*<sup>-/-</sup> HSCs do not exhibit increased rates of apoptosis, but an accrued proliferative capacity (Liu *et al*, 2010). The deletion of *Rb1cc1* in murine neuronal stem cells (NSCs) also causes a functional impairment that compromises postnatal neuronal differentiation (Wang *et al*, 2013). However, this effect appears to stem from the failure of murine *Rb1cc1*<sup>-/-</sup> HNCs to control redox homeostasis, resulting in the activation of a tumor protein p53 (TP53)-dependent apoptotic response (Wang *et al*, 2013). Finally, *Becn1*<sup>+/-</sup> mice display an expansion of progenitor-like mammary epithelial cells (Cicchini *et al*, 2014). Of note, autophagy also appears to be required for the preservation of normal stem cell compartments in the human system. Indeed, human hematopoietic, dermal, and epidermal stem cells transfected with a short-hairpin RNA (shRNA) specific for ATG5 lose their ability to self-renew while differentiating into neutrophils, fibroblasts, and keratinocytes, respectively (Salemi *et al*, 2012).

It has been proposed that autophagy contributes to oncogene-induced cell death or oncogene-induced senescence, two fundamental oncosuppressive mechanisms. The activation of various oncogenes imposes indeed a significant stress on healthy cells, a situation that is normally aborted through the execution of a cell death program (Elgendy *et al*, 2011), or upon the establishment of permanent proliferative arrest (cell senescence) that engages the innate arm of the immune system (Iannello *et al*, 2013). The partial depletion of ATG5, ATG7 or BECN1 limited the demise of human ovarian cancer cells pharmacologically stimulated to express *HRAS*<sup>G12V</sup> from an inducible construct (Elgendy *et al*, 2011). Similarly, shRNAs specific for ATG5 or ATG7 prevented oncogene-induced senescence in primary human melanocytes or human diploid fibroblasts (HDFs) expressing *BRAF*<sup>V600E</sup> or *HRAS*<sup>G12V</sup> (Young *et al*, 2009; Liu *et al*, 2013a). Accordingly, the overexpression of the ULK1 homolog ULK3 was sufficient to limit the proliferative potential of HDFs while promoting autophagy (Young *et al*, 2009). Moreover, both pharmacological inhibitors of autophagy and small-interfering RNAs targeting ATG5, ATG7 or BECN1 prevented spontaneous senescence in HDFs while preventing the degradation of an endogenous, dominant-negative TP53 variant (Horikawa *et al*, 2014). Finally, ectopic ATG5 expression reduced the colony-forming ability of melanoma cell lines normally characterized by low ATG5 levels, an effect that could be reproduced by the administration of autophagy inducers (Liu *et al*, 2013a). Apparently at odds with these results, *HRAS*<sup>G12V</sup> fails to induce senescence in mouse embryonic fibroblasts (MEFs) lacking transformation-related protein 53 binding protein 2 (Trp53bp2), correlating with the stabilization of Atg5/Atg12 complexes and consequent upregulation of the autophagic flux. In line with this notion, ectopic expression of Atg5 prevented Trp53bp2-sufficient MEFs from entering senescence upon overexpression of *HRAS*<sup>G12V</sup> (Wang *et al*, 2012b). Thus, while in some cells autophagy appears to inhibit malignant transformation by favoring oncogene-induced senescence, this may not be a general mechanism of autophagy-mediated oncosuppression.

It has been suggested that autophagy is involved in the degradation of oncogenic proteins, including mutant (but not wild-type) TP53 (Rodriguez *et al*, 2012; Choudhury *et al*, 2013; Garufi *et al*,

2014), p62 (Duran *et al*, 2008; Mathew *et al*, 2009; Ling *et al*, 2012), PML-RARA (Isakson *et al*, 2010; Wang *et al*, 2011), and BCR-ABL1 (Goussetis *et al*, 2012). Mutant TP53 often accumulates in neoplastic cells and operates as a dominant-negative factor, thereby interfering with the oncosuppressive function of the wild-type protein (de Vries *et al*, 2002). Cancer cells depleted of ULK1, BECN1 or ATG5 tend to accumulate increased amounts of mutant TP53, whereas the transgene-driven overexpression of BECN1 or ATG5 results in mutant TP53 depletion (Choudhury *et al*, 2013). Such an autophagy-dependent degradation of mutant TP53 would therefore restore the ability of wild-type TP53 to inhibit malignant transformation, at least in some settings. It is worth noting that both ATG5 and ATG7 have been involved in the regulation of TP53-dependent adaptive responses to stress (Lee *et al*, 2012; Salemi *et al*, 2012). However, this activity appears to be independent of autophagy, at least in the case of ATG7 (Lee *et al*, 2012). Interestingly, p62 itself has been ascribed with potentially oncogenic functions, including a key role in the transduction of RAS-elicited signals as well as in the activation of a feedforward loop involving the cytoprotective transcription factor NF- $\kappa$ B driven by oncogenic stress (Duran *et al*, 2008; Mathew *et al*, 2009; Takamura *et al*, 2011; Ling *et al*, 2012). Autophagy may therefore inhibit oncogenesis by limiting p62 availability (Mathew *et al*, 2009), at least in some settings.

The t(9;22)(q34;q11) translocation is found in about 90% of chronic myeloid leukemia patients, resulting in the synthesis of a fusion protein that involves breakpoint cluster region (BCR) and ABL proto-oncogene 1 (ABL1) (Ben-Neriah *et al*, 1986). BCR-ABL1 is a constitutively active kinase and is etiologically involved in leukemogenesis, as demonstrated by the outstanding clinical success of imatinib mesylate, a BCR-ABL1-targeting kinase inhibitor (Druker *et al*, 2001). Arsenic trioxide, a chemotherapeutic agent commonly employed against various forms of leukemia, appears to trigger the p62-dependent and cathepsin B-dependent degradation of BCR-ABL1 in leukemic progenitors (Goussetis *et al*, 2012). In line with this notion, the pharmacological or genetic inhibition of autophagy or cathepsin B reportedly limits the antileukemic potential of arsenic trioxide (Goussetis *et al*, 2012). The t(15;17)(q22;q21) translocation can be documented in 95% of promyelocytic leukemia cases, resulting in the expression of a chimera that involves promyelocytic leukemia (PML) and retinoic acid receptor, alpha (RARA) (Goddard *et al*, 1991). PML-RARA blocks normal retinoic acid-dependent myeloid differentiation, *de facto* driving leukemogenesis (Rousselot *et al*, 1994). Patients expressing PML-RARA generally benefit from the administration of all-*trans* retinoic acid (ATRA), resulting in PML-RARA degradation and restored myeloid differentiation (Wang *et al*, 2011). Pharmacological and genetic evidence suggests that autophagy is implicated in both ATRA- and arsenic trioxide-driven PML-RARA degradation (Isakson *et al*, 2010; Wang *et al*, 2011). Further experimentation is required to understand whether autophagy degrades potentially oncogenic proteins in cells not exposed to chemotherapeutic agents.

Autophagy is implicated in immune responses that prevent the establishment and proliferation of malignant cells (Ma *et al*, 2013). At least in some circumstances, dying malignant cells are capable of recruiting antigen-presenting cells (APCs) and other cellular components of the immune system, resulting in the elicitation of innate and/or adaptive antitumor immune responses (Deretic *et al*, 2013; Kroemer *et al*, 2013). On the one hand, autophagic responses are

required for dying neoplastic cells to release ATP in optimal amounts, which not only recruits APCs through purinergic receptor P2Y, G-protein coupled, 2 (P2RY2), but also activates them to release immunostimulatory chemokines through purinergic receptor P2X, ligand-gated ion channel, 7 (P2RX7) (Michaud *et al*, 2011). On the other hand, autophagy in immune cells is implicated in several steps of both adaptive and innate immune responses (Ma *et al*, 2013). Thus, both cancer cell-intrinsic and systemic defects in autophagy may prevent the host immune system to properly recognize and eliminate pre-malignant and malignant cells.

Autophagy mediates potent anti-inflammatory effects (Deretic *et al*, 2013). At least in some cases, malignant transformation is stimulated by an inflammatory microenvironment, which contains high amounts of potentially genotoxic ROS as well as various mitogenic cytokines (Coussens *et al*, 2013). Proficient autophagic responses limit inflammation as: (1) they efficiently dispose of the so-called inflammasomes (the supramolecular platforms that are responsible for the maturation and secretion of pro-inflammatory interleukin-1 $\beta$  and interleukin-18), as well as damaged mitochondria, which would otherwise release endogenous inflammasome activators (Nakahira *et al*, 2011; Zitvogel *et al*, 2012); (2) they are linked to the inhibition of pro-inflammatory signals delivered by some pattern recognition receptors, such as RIG-I-like receptors (Jounai *et al*, 2007); (3) they limit the abundance of B-cell CLL/lymphoma 10 (BCL10), a protein involved in pro-inflammatory NF- $\kappa$ B signaling (Paul *et al*, 2012); (4) they are connected to the inhibition of transmembrane protein 173 (TM173, best known as STING), a pattern recognition receptor involved in the delivery of pro-inflammatory cues in response to cytosolic nucleic acids (Saitoh *et al*, 2009).

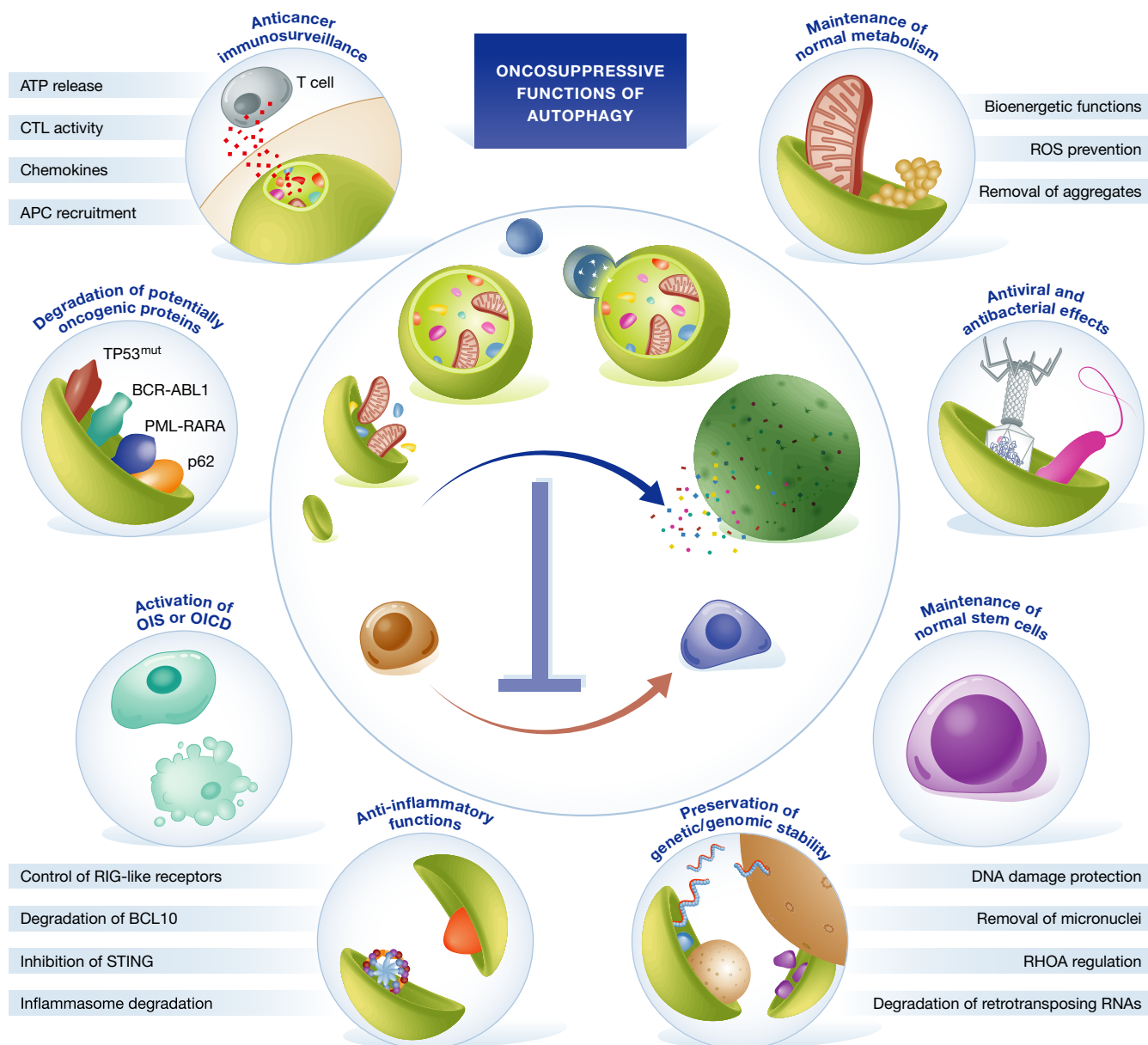
Finally, autophagy may suppress carcinogenesis owing to its key role in the first line of defense against viral and bacterial infection (Deretic *et al*, 2013). Indeed, several potentially carcinogenic pathogens potentially activate autophagy upon infection. These pathogens include hepatitis B virus (which promotes hepatocellular carcinoma), human herpesvirus 8 (which causes Kaposi's sarcoma and contributes to the pathogenesis of primary effusion lymphoma and multicentric Castleman's disease), human papillomavirus type 16 and 18 (HPV-16 and HPV-18, which cause cervical carcinoma), Epstein-Barr virus and *Helicobacter pylori* (both of which are associated with gastric carcinoma), *Streptococcus bovis* (which causes colorectal carcinoma), *Salmonella enterica* (which is associated with an increased incidence of Crohn's disease, hence sustaining colorectal carcinogenesis, and gallbladder carcinoma), as well as *Chlamydia pneumoniae* (an etiological determinant in some forms of lung cancer) (Nakagawa *et al*, 2004; Travassos *et al*, 2010; Yasir *et al*, 2011; Conway *et al*, 2013; Griffin *et al*, 2013; Zhang *et al*, 2014). Such a xenophagic response is required for the rapid clearance of intracellular pathogens as well as for the stimulation of pathogen-specific immune responses (Deretic *et al*, 2013; Ma *et al*, 2013). Accordingly, epithelial cells bearing molecular defects in the autophagic machinery, such as those provoked by Crohn's disease-associated point mutations in *ATG16L1* and nucleotide-binding oligomerization domain containing 2 (*NOD2*) (Lassen *et al*, 2014), are more susceptible to infection by intracellular pathogens than their wild-type counterparts. In line with this notion, reduced levels of autophagic markers including BECN1 have recently been correlated with HPV-16 and HPV-18 infection in a cohort of cervical

carcinoma patients (Wang *et al*, 2014). Thus, autophagy may exert oncosuppressive effects also by virtue of its antiviral and antibacterial activity.

Taken together, these observations suggest that autophagy prevents malignant transformation by preserving both cellular and organismal homeostasis in conditions that pose a risk for oncogenesis (Fig 2).

## Oncoproteins, oncosuppressor proteins and autophagy

In agreement with the oncosuppressive activity of autophagy, several oncoproteins, that is, proteins that drive malignant transformation upon overexpression- or mutation-dependent hyperactivation, inhibit autophagic responses (Maiuri *et al*, 2009). Along similar lines, many *bona fide* oncosuppressor proteins, that is,



**Figure 2. Oncosuppressive functions of autophagy.**

Autophagy has been proposed to suppress malignant transformation by several mechanisms, including: (1) the preservation of genetic/genomic stability; (2) the disposal of endogenous sources of potentially mutagenic reactive oxygen species (ROS); (3) the maintenance of normal bioenergetic functions; (4) the degradation of oncogenic proteins; (5) cell-endogenous antiviral and antibacterial effects; (6) the optimal activation of oncogene-induced senescence (OIS) and oncogene-induced cell death (OICD); (7) the maintenance of a normal stem cell compartment; (8) multipronged anti-inflammatory functions; and (9) a key role in the elicitation and execution of anticancer immunosurveillance. ABL1, ABL proto-oncogene 1; APC, antigen-presenting cell; BCL10, B-cell CLL/lymphoma 10; BCR, breakpoint cluster region; CTL, cytotoxic T lymphocyte; TP53<sup>mut</sup>, mutant tumor protein p53; PML, promyelocytic leukemia; RARA, retinoic acid receptor, alpha; RHOA, *ras* homology family member.

proteins that are inactivated or lost in the course of oncogenesis, stimulate autophagy (Morselli *et al*, 2011) (Table 1).

### Oncoproteins

BECN1 is inhibited upon sequestration by several members of the Bcl-2 protein family, including B-cell CLL/lymphoma 2 (BCL2) and BCL2-like 1 (BCL2L1, best known as BCL-X<sub>L</sub>) (Patingre *et al*, 2005; Maiuri *et al*, 2007b). These proteins are overexpressed by a wide variety of hematological and solid tumors, an event that consistently reduces the sensitivity of neoplastic cells to lethal cues of either cell-intrinsic or environmental origin (Kang & Reynolds, 2009; Anderson *et al*, 2014). Besides inhibiting BECN1, BCL-X<sub>L</sub> (but not BCL2) also suppresses the ability of phosphoglycerate mutase family member 5 (PGAM5) to dephosphorylate FUN14 domain containing 1 (FUNDC1) on S13, hence suppressing mitophagy (Wu *et al*, 2014). Interestingly, the robust oncogenic activity of Bcl-2 proteins may also reflect their metabolic and autophagy-inhibitory activity (Patingre & Levine, 2006; Oh *et al*, 2011; Wei *et al*, 2013; Green *et al*, 2014).

Various oncoproteins stimulate malignant transformation by virtue of their ability to inactivate TP53. These include the human protein MDM2, which is overexpressed by a wide panel of human tumors as a result of gene amplification (Oliner *et al*, 1992), as well as the early HPV-16 protein E6, which is etiologically involved in the pathogenesis of human cervical carcinoma (de Freitas *et al*, 2014). Both MDM2 and E6 operate as E3 ligases and target TP53 to proteasomal degradation (Hock & Vousden, 2014), hence suppressing its ability to promote autophagy (and regulated cell death) in response to stress (Vousden & Lane, 2007; Hanning *et al*, 2013; Pietrocola *et al*, 2013). Also E7, another early HPV-16 gene product that is required for viral carcinogenesis because it inhibits the oncosuppressor protein retinoblastoma 1 (RB1) (Dyson *et al*, 1989), has been shown to suppress autophagic responses (Hanning *et al*, 2013). The ability of E7 to inhibit RB1 may explain its autophagy-suppressing functions, at least in specific settings (see below) (Jiang *et al*, 2010).

A consistent fraction of human neoplasms is characterized by the hyperactivation of receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR) and *v-erb-b2* avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2, best known as HER2) or downstream signal transducers, including SRC, *v-akt* murine thymoma viral oncogene homolog 1 (AKT1), class I phosphatidylinositol 3-kinases (PI3Ks) and phosphoinositide-dependent protein kinase 1 (PDK1) (Slamon *et al*, 1987; Irby *et al*, 1999; Shayesteh *et al*, 1999; Ma *et al*, 2000; Paez *et al*, 2004; Stephens *et al*, 2004; Carpten *et al*, 2007; Sen & Johnson, 2011; Chinen *et al*, 2014). According to current views, this promotes neoplastic transformation because it allows cells to proliferate in the absence of growth factors and it increases their resistance to adverse microenvironmental conditions (Manning & Cantley, 2007; Laplante & Sabatini, 2012). In addition, the signal transduction cascades elicited by RTKs often impinge, either directly or upon the engagement of additional signaling modules, on the activation of MTORC1, *de facto* inhibiting autophagy (Laplante & Sabatini, 2012; Lozy *et al*, 2014). Of note, AKT1 reportedly suppresses autophagic responses not only by catalyzing the inactivating phosphorylation of the MTOR-repressing tumor sclerosis (TSC) complex, but also by phosphorylating and hence inhibiting BECN1 (Wang *et al*, 2012a), and perhaps by promoting the ability of TP53 to block autophagy in baseline or near-to-baseline

conditions via a X-linked inhibitor of apoptosis (XIAP)- and MDM2-dependent signal transduction cascade (Huang *et al*, 2013). In line with this notion, although XIAP does not behave as a conventional oncoprotein, several human neoplasms express increased XIAP levels (Schimmer *et al*, 2006). Moreover, SRC not only suppresses autophagic responses by activating MTORC1 via the PI3K/PDK1/AKT1 signaling module (Laplante & Sabatini, 2012), but also inhibits mitophagy by catalyzing the inactivating phosphorylation of FUNDC1 on Y18 (Liu *et al*, 2012b). Finally, some RTKs such as EGFR inhibit autophagy in an MTORC1-independent manner, by catalyzing the inactivating phosphorylation of BECN1 on tyrosine residues (Wei *et al*, 2013).

At least three members of the RAS GTPase family, that is, Harvey rat sarcoma viral oncogene homolog (HRAS), Kirsten rat sarcoma viral oncogene homolog (KRAS) and neuroblastoma RAS viral (*v-ras*) oncogene homolog (NRAS), are etiologically involved in several paradigms of malignant transformation, either as a result of somatic mutations or upon the hyperactivation of upstream signal transducers (including RTKs) (Shaw & Cantley, 2006). Besides delivering robust mitogenic signals, hyperactivated RAS engages the PI3K/PDK1/AKT1 signaling cascade, hence potently suppressing autophagic responses (in both mammals and *Drosophila*) (Furuta *et al*, 2004; Denton *et al*, 2012a; Laplante & Sabatini, 2012). However, RAS also transactivates p62 through NF- $\kappa$ B, hence igniting a p62-dependent feedforward signaling loop with pro-autophagic and oncogenic effects accompanied by sustained NF- $\kappa$ B activation (Duran *et al*, 2008; Ling *et al*, 2012). Along similar lines, RAS activates mitogen-activated protein kinase 8 (MAPK8, best known as JNK1) and nuclear factor, erythroid 2-like 2 (NFE2L2, best known as NRF2), thereby promoting autophagy via the JNK1-dependent phosphorylation of BCL2 (and the consequent derepression of BECN1), and the NRF2-dependent transactivation of genes encoding several autophagy receptors, including p62 and calcium binding and coiled-coil domain 2 (CALCOCO2, best known as NDP52) (Wei *et al*, 2008; Jain *et al*, 2010; DeNicola *et al*, 2011; Lock *et al*, 2011; Jo *et al*, 2014). Interestingly, the oncogene-driven activation of NRF2 may engage a pro-autophagic feedforward loop, stemming from the autophagy-dependent degradation of kelch-like ECH-associated protein 1 (KEAP1, the major negative regulator of NRF2) (Taguchi *et al*, 2012; Bae *et al*, 2013), as well as from the NRF2-activating phosphorylation of p62 (Inami *et al*, 2011; Ichimura *et al*, 2013). Autophagic responses are indeed recovered along with tumor progression in various models of KRAS-driven oncogenesis (see below) (Guo *et al*, 2013a; Rosenfeldt *et al*, 2013; Rao *et al*, 2014). Another member of the RAS family, that is, Ras homolog enriched in brain (RHEB), is often overexpressed due to genetic amplification in human prostate carcinoma (Nardella *et al*, 2008). RHEB suppresses autophagy as it operates as a direct activator of MTORC1 downstream of AKT1 (Inoki *et al*, 2003; Nardella *et al*, 2008).

Hyperactivating mutations in *B-Raf* proto-oncogene, serine/threonine kinase (BRAF), in particular the C1799T substitution (generating BRAF<sup>V600E</sup>), are a common finding in biopsies from human melanoma patients (Davies *et al*, 2002) and have recently been associated with various forms of histiocytosis (a pseudomalignant condition affecting macrophages and dendritic cells) (Berres *et al*, 2014; Hervier *et al*, 2014). At odds with its wild-type counterpart, BRAF<sup>V600E</sup> engages the mitogenic MAPK/ERK signaling pathway



**Table 1. Oncoproteins, oncosuppressor proteins and autophagy.**

Protein	Function(s)	Link(s) to cancer	Link(s) to autophagy	Reference
<i>Oncoproteins</i>				
AKT1	Serine/threonine kinase	Hyperactivated or overexpressed in various neoplasms	Inhibits BECN1 and activates MTORC1  Stimulates autophagy via XIAP	Carpent <i>et al</i> (2007); Laplante & Sabatini (2012); Wang <i>et al</i> (2012a); Huang <i>et al</i> (2013)
BCL2 BCL-X <sub>L</sub>	Anti-apoptotic Bcl-2 family members	Overexpressed in various hematological and solid tumors	Sequester BECN1 in inactive complexes  BCL-X <sub>L</sub> inhibits mitophagy mediated by FUNDC1	Pattingre <i>et al</i> (2005); Maiuri <i>et al</i> (2007b); Kang & Reynolds (2009); Anderson <i>et al</i> (2014); Wu <i>et al</i> (2014)
BRAF	Serine/threonine kinase	Mutated in melanoma and various histiocytoses	Activates MTORC1 via ERK\TSC2\RHEB signaling  BRAF hyperactivation promotes ER stress, in turn triggering autophagy	Davies <i>et al</i> (2002); Sharma <i>et al</i> (2006); Berres <i>et al</i> (2014); Corazzari <i>et al</i> (2014); Hervier <i>et al</i> (2014); Ma <i>et al</i> (2014)
E6	E3 ubiquitin ligase	Etiological factor in HPV-associated cancers	Inhibits TP53	Hanning <i>et al</i> (2013); de Freitas <i>et al</i> (2014); Hock & Vousden (2014)
E7	RB1 inhibitor	Etiological factor in HPV-associated cancers	Suppresses autophagy, perhaps as a result of RB1 inhibition	Jiang <i>et al</i> (2010); Hanning <i>et al</i> (2013); de Freitas <i>et al</i> (2014)
HIF-1	Transcription factor	Overexpressed in various tumors	Promotes mitophagy by transactivating <i>BNIP3</i> and <i>BNIP3L</i>	Tracy <i>et al</i> (2007); Zhang <i>et al</i> (2008); Bellot <i>et al</i> (2009); Luo <i>et al</i> (2009); Wilkinson <i>et al</i> (2009)
HRAS KRAS NRAS	Small GTP-binding proteins	Hyperactivated or overexpressed in various neoplasms	Activate MTORC1 via PI3K signaling  Derepress BECN1 upon the JNK1-mediated phosphorylation of BCL2  Promote the NRF2-dependent synthesis of p62 and NDP52	Furuta <i>et al</i> (2004); Shaw & Cantley (2006); Wei <i>et al</i> (2008); DeNicola <i>et al</i> (2011); Laplante & Sabatini (2012)
MDM2	E3 ubiquitin ligase	Overexpressed in various neoplasms	Inhibits TP53	Oliner <i>et al</i> (1992); Hock & Vousden (2014)
MYC MYCL MYCN	Transcription factors	Hyperactivated or overexpressed in various neoplasms	Inhibit autophagy upon 4EBP1 expression  Transactivate <i>MAPK8</i> , coding for the BECN1 derepressor JNK1  Truncated MYC promotes autophagy independent of transcription  MYC hyperactivation promotes ER stress, in turn triggering autophagy	Dalla-Favera <i>et al</i> (1982); Balakumaran <i>et al</i> (2009); Dang (2012); Toh <i>et al</i> (2013); Conacci-Sorrell <i>et al</i> (2014)
PDPK1	Serine/threonine kinase	Hyperactivated in colorectal neoplasms	Activates MTORC1 via AKT1\TSC2\RHEB signaling	Laplante & Sabatini (2012); Chinen <i>et al</i> (2014)
PI3K	Lipid kinase	Hyperactivated in various neoplasms	Activates MTORC1 via AKT1\TSC2\RHEB signaling	Shayesteh <i>et al</i> (1999); Ma <i>et al</i> (2000); Laplante & Sabatini (2012)
RHEB	Small GTP-binding protein	Overexpressed in prostate carcinoma	Activates MTORC1	Inoki <i>et al</i> (2003); Nardella <i>et al</i> (2008)

Table 1 (continued)

Protein	Function(s)	Link(s) to cancer	Link(s) to autophagy	Reference
RTKs	Tyrosine kinases	Hyperactivated or overexpressed in various neoplasms	Activate MTORC1 via PI3K signaling  EGFR phosphorylates BECN1, hence inactivating it	Slamon <i>et al</i> (1987); Paez <i>et al</i> (2004); Stephens <i>et al</i> (2004); Laplante & Sabatini (2012); Wei <i>et al</i> (2013); Lozy <i>et al</i> (2014)
SRC	Non-receptor tyrosine kinase	Hyperactivated in various cancers	Activates MTORC1 via PI3K signaling  Phosphorylates FUNDC1, hence inactivating it	Irby <i>et al</i> (1999); Sen & Johnson (2011); Liu <i>et al</i> (2012b)
XIAP	E3 ubiquitin ligase	Overexpressed in various tumors	Inhibits the autophagy-blocking activity of cytoplasmic TP53 (?)	Schimmer <i>et al</i> (2006); Huang <i>et al</i> (2013)
<i>Oncosuppressor proteins</i>				
AMBRA1	Component of class III PI3K complex	Mutated in endometrial, colorectal and urinary tract neoplasms	Key factor for canonical autophagy	Codogno <i>et al</i> (2012); Cianfanelli <i>et al</i> (2015)
ATG5	E3 ubiquitin ligase	Downregulated in melanoma	Key factor for canonical autophagy	Codogno <i>et al</i> (2012); Liu <i>et al</i> (2013a)
BECN1	Component of class III PI3K complex	Monoallelically deleted or downregulated in various solid tumors	Key factor for canonical autophagy	Liang <i>et al</i> (1999); Qu <i>et al</i> (2003); Miracco <i>et al</i> (2007); Codogno <i>et al</i> (2012); Laddha <i>et al</i> (2014)
BH3-only proteins	Pro-apoptotic Bcl-2 family members	Downregulated in various hematological and solid tumors	Derepress BECN1 by displacing it from BCL2 and BCL-X <sub>L</sub>	Ahn <i>et al</i> (2007); Maiuri <i>et al</i> (2007a); Maiuri <i>et al</i> (2007b)
BIF1	Component of class III PI3K complex	Downregulated in colorectal carcinoma	UVRAG interactor	Takahashi <i>et al</i> (2007); Coppola <i>et al</i> (2008); Takahashi <i>et al</i> (2013)
DAPK1	Serine/threonine kinase	Downregulated in various solid tumors	Derepresses BECN1 by displacing it from BCL2  Boosts a potentially self-amplifying p19 <sup>ARF</sup> /TP53 response	Raveh <i>et al</i> (2001); Martoriati <i>et al</i> (2005); Christoph <i>et al</i> (2007); Zalckvar <i>et al</i> (2009)
DIRAS3	GTP-binding protein	Downregulated in breast and ovarian carcinoma	Inhibits MTORC1 by antagonizing PI3K signaling	Yu <i>et al</i> (1999); Feng <i>et al</i> (2008); Lu <i>et al</i> (2008)
DRAM1	Lysosomal protein	Downregulated in many tumors as a result of TP53 inactivation	Involved in TP53-dependent autophagy	Crighton <i>et al</i> (2006); Leroy <i>et al</i> (2014)
FIP200	Multifunctional protein	Affected by truncating mutations in breast cancer	Component of the ULK1-ATG13-ATG101 complex	Chano <i>et al</i> (2002); Hara <i>et al</i> (2008)
FOXO1	Transcription factor	Mutated in DLBCL	Interacts with ATG7 in the course of stress-induced autophagy	Huang <i>et al</i> (2005); Zhao <i>et al</i> (2010)
LKB1	Serine/threonine kinase	Germline mutations cause Peutz–Jeghers's syndrome  Mutated in a proportion of SCCs and lung carcinomas	Activates the TSC complex via AMPK	Hemminki <i>et al</i> (1998); Shaw <i>et al</i> (2004); Ji <i>et al</i> (2007)
NF1	GTPase	Germline mutations cause type I neurofibromatosis	Activates the TSC complex by antagonizing RAS signaling	Fountain <i>et al</i> (1989); Xu <i>et al</i> (1990); Johannessen <i>et al</i> (2005)

Table 1 (continued)

Protein	Function(s)	Link(s) to cancer	Link(s) to autophagy	Reference
RB1	Transcription factor	Germline mutations cause hereditary retinoblastoma  Mutated in various solid tumors	Stimulates autophagy, perhaps upon BCL2 downregulation and consequent BECN1 derepression	Friend <i>et al</i> (1986); Cryns <i>et al</i> (1994); Gomez-Manzano <i>et al</i> (2001); Jiang <i>et al</i> (2010)
p19 <sup>ARF</sup>	MDM2 inhibitor	Mutated or deleted in several hematological and solid tumor	Stimulates autophagy via TP53-dependent and -independent mechanisms	Faderl <i>et al</i> (1999); Abida & Gu (2008); Pimkina <i>et al</i> (2009)
PTEN	Lipid phosphatase	Germline mutations cause the so-called PHTS  Sporadically mutated in a large fraction of neoplasms	Inhibits MTORC1 by antagonizing PI3K signaling	Liaw <i>et al</i> (1997); Marsh <i>et al</i> (1997); Arico <i>et al</i> (2001); Sansal & Sellers (2004)
smARF	Alternative CDKN2A product	Mutated or deleted in several hematological and solid tumors	Derepresses BECN1 by displacing it from BCL-X <sub>L</sub>	Faderl <i>et al</i> (1999); Reef <i>et al</i> (2006); Pimkina <i>et al</i> (2009)
TP53	Transcription factor	Germline mutations cause the Li-Fraumeni syndrome  Mutated or inactivated in > 50% of human malignancies	In physiological conditions, inhibits autophagy via non-transcriptional, cytoplasmic mechanisms  In response to stress, transactivate a panel of genes involved in adaptive responses, including autophagy	Malkin <i>et al</i> (1990); Moll <i>et al</i> (2005); Riley <i>et al</i> (2008); Maiuri <i>et al</i> (2010); Pietrocola <i>et al</i> (2013); Leroy <i>et al</i> (2014); McBride <i>et al</i> (2014)
TSC1 TSC2	GTPases	Germline mutations cause TSC	The TSC complex inhibits MTORC1 via RHEB	van Slegtenhorst <i>et al</i> (1997); Inoki <i>et al</i> (2002)
UVRAG	Component of class III PI3K complex	Monoallelically deleted in gastric and colorectal cancers	Positive modulator of BECN1	Liang <i>et al</i> (2006); Kim <i>et al</i> (2008); Zhao <i>et al</i> (2012)
VHL	E3 ubiquitin ligase	Germline mutations cause the von Hippel-Lindau syndrome  Sporadically mutated in a large fraction of RCC	Inhibits LC3B and TRPM3 via miR-204  Inhibits the HIF-1-dependent synthesis of BNIP3 and BNIP3L	Seizinger <i>et al</i> (1988); Shuin <i>et al</i> (1994); Tracy <i>et al</i> (2007); Zhang <i>et al</i> (2008); Bellot <i>et al</i> (2009); Wilkinson <i>et al</i> (2009); Mikhaylova <i>et al</i> (2012); Hall <i>et al</i> (2014)

AKT1, *v-akt* murine thymoma viral oncogene homolog 1; AMBRA1, autophagy/beclin-1 regulator 1; AMPK, 5'-AMP-activated protein kinase; BCL2, B-cell CLL/lymphoma 2; BECN1, Beclin 1; BNIP3, BCL2/adenovirus E1B 19 kDa interacting protein 3; BNIP3L, BNIP3-like; BRAF, *B-Raf* proto-oncogene, serine/threonine kinase, CDKN2A, cyclin-dependent kinase inhibitor 2A; DAPK1, death-associated protein kinase 1; DIRAS3, DIRAS family, GTP-binding RAS-like 3; DRAM1, DNA-damage-regulated autophagy modulator 1; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; FOXO1, forkhead box O1; FUNDC1, FUN14 domain containing 1; HIF-1, hypoxia-inducible factor 1; HPV, human papillomavirus; HRAS, Harvey rat sarcoma viral oncogene homolog; KRAS, Kirsten rat sarcoma viral oncogene homolog; MTORC1, mechanistic target of rapamycin complex 1; MYC, *v-myc* avian myelocytomatosis viral oncogene homolog; MYCL, *v-myc* avian myelocytomatosis viral oncogene lung carcinoma-derived homolog; MYCN, *v-myc* avian myelocytomatosis viral oncogene neuroblastoma derived homolog; NF1, neurofibromin 1; NRAS, neuroblastoma RAS viral (*v-ras*) oncogene homolog; PDPK1, phosphoinositide-dependent protein kinase 1; PHTS, PTEN hamartoma tumor syndrome; PI3K, phosphoinositide-3-kinase; PTEN, phosphatase and tensin homolog; RB1, retinoblastoma 1; RCC, renal cell carcinoma; RHEB, Ras homolog enriched in brain; RTK, receptor tyrosine kinase; SCC, squamous cell carcinoma; TP53, tumor protein p53; TRPM3, transient receptor potential nonselective cation channel, subfamily M, member 3; TSC, tuberous sclerosis; ULK1, unc-51-like autophagy-activating kinase 1; UVRAG, UV radiation resistance associated; VHL, von Hippel-Lindau tumor suppressor; XIAP, X-linked inhibitor of apoptosis.

even in the absence of RTK-emanated and RAS-transduced signals, *de facto* rendering cell proliferation independent of extracellular growth factor availability (Sharma *et al*, 2006). Activated ERK phosphorylates TSC2, hence inhibiting autophagy as a consequence of mTORC1 activation (Ma *et al*, 2005). In addition, constitutive signaling via the BRAF axis results in a chronic state of ER stress that triggers an adaptive autophagic response required for the survival of transformed cells and, at least in some settings, sustaining their resistance to pharmacological BRAF inhibitors (Corazzari *et al*, 2014; Ma *et al*, 2014). This may explain, at least in part, the sensitivity of established *BRAF*<sup>V600E</sup>-driven lung adenocarcinomas to the deletion of *Atg7* (see below) (Strohecker *et al*, 2013).

*v-myc* avian myelocytomatosis viral oncogene homolog (MYC), *v-myc* avian myelocytomatosis viral oncogene lung carcinoma-derived homolog (MYCL) and *v-myc* avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN) are the major effectors of the mitogenic MAPK/ERK signal transduction cascade (Dang, 2012). *MYC*, *MYCL* and *MYCN* are affected by various mutational events in a wide panel of human malignancies (Dang, 2012). These include point mutations as well as larger genetic rearrangements such as the t(8;14)(q24;q32) translocation, which is etiologically associated with Burkitt's lymphoma (Dalla-Favera *et al*, 1982). Besides overriding the microenvironmental control on proliferation, the hyperactivation of MYC family members has been

linked to suppressed autophagy as a consequence of eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1, best known as 4EBP1) overexpression (Balakumaran *et al*, 2009). Apparently at odds with this notion, MYC depletion reportedly inhibits the formation of autophagosomes, possibly as a consequence of reduced JNK1 expression and consequent BCL2 phosphorylation (Toh *et al*, 2013). In addition, MYC-driven oncogenesis resembles its BRAF-driven counterparts in that it causes a chronic condition of ER stress that initiates compensatory adaptive responses (Hart *et al*, 2012). Moreover, it seems that colorectal cancer cells exposed to nutrient deprivation and hypoxia accumulate a transcriptionally inactive, cytoplasmic, calpain-derived cleavage product of MYC that not only supports their survival by triggering autophagy, but also favors other facets of tumor progression, including anchorage-independent growth and genomic instability (Conacci-Sorrell *et al*, 2014).

In summary, the net effect of RAS hyperactivation and its consequences on autophagy exhibit (at least some degree of) context dependency. This apparent discrepancy may reflect the presence/absence of genetic abnormalities other than the activation of a single oncogene. Irrespective of this hitherto unexplored possibility, autophagy appears to be restored and to be required for optimal tumor progression in rodent models of *KRAS*-, *BRAF*- and *MYC*-driven oncogenesis, perhaps as a compensatory response to oncogenic stress that ensues, rather than accompanies, transformation (Guo *et al*, 2013a; Rosenfeldt *et al*, 2013; Strohecker *et al*, 2013; Conacci-Sorrell *et al*, 2014; Rao *et al*, 2014). The molecular mechanisms that are responsible for the reconstitution of autophagic responses in these settings have not yet been elucidated.

#### Oncosuppressor proteins

The Li–Fraumeni syndrome, a dominantly inherited disorder characterized by the early-onset of various tumors (including bone and soft tissue sarcomas, breast carcinomas, brain cancers and acute leukemias), is caused by germline mutations in *TP53* (Malkin *et al*, 1990; McBride *et al*, 2014). Moreover, the *TP53* system is inactivated as a consequence of somatic mutations or genetic/epigenetic events affecting its regulators, such as *MDM2* (see above), in more than 50% of sporadic human malignancies (Leroy *et al*, 2014). Besides engaging the cell death machinery in cells bearing irreparable molecular defects (mainly DNA damage), via transcriptional as well as transcription-independent mechanisms (Moll *et al*, 2005; Riley *et al*, 2008), stress-stabilized *TP53* can transactivate various genes involved in autophagic responses (Maiuri *et al*, 2010; Pietrocola *et al*, 2013). These include, but are not limited to, the genes coding for the  $\beta 1$  and  $\beta 2$  subunits of *AMPK* (i.e., *PRKAB1*, *PRKAB2*) (Feng *et al*, 2007), the gene coding for the *AMPK* activators sestrin 1 (i.e., *SESN1*) and *SESN2* (Budanov *et al*, 2004; Budanov & Karin, 2008), genes encoding various BH3-only proteins (i.e., *BAD*, *BNIP3*) and death-associated protein kinase 1 (i.e., *DAPK1*), all of which stimulate autophagy by favoring the displacement of *BECN1* from inhibitory interactions with *BCL2* and *BCL-X<sub>L</sub>* (Martoriati *et al*, 2005; Feng *et al*, 2007; Maiuri *et al*, 2007a; Maiuri *et al*, 2007b; Zalckvar *et al*, 2009), as well as the gene coding for DNA-damage-regulated autophagy modulator 1 (i.e., *DRAM1*), a lysosomal protein that sustains *TP53*-dependent autophagic responses via an unknown mechanism (Crighton *et al*, 2006). The gene coding for the upstream activator of *TP53* *p19<sup>ARF</sup>* (i.e., *CDKN2A*) is also mutated or lost in several solid and hematological malignancies (Faderl *et al*, 1999). Full-length

*p19<sup>ARF</sup>* promotes autophagy via *TP53*- as well as *TP53*-independent mechanisms (Abida & Gu, 2008). Moreover, a short mitochondrial isoform of *p19<sup>ARF</sup>* commonly known as *smARF* stimulates autophagic responses by displacing *BECN1* from *BCL-X<sub>L</sub>* (Reef *et al*, 2006). Interestingly, at least part of the autophagy-promoting functions of *DAPK1* may result from its ability to initiate a potentially self-amplifying *p19<sup>ARF</sup>*/*TP53* signal transduction cascade (Raveh *et al*, 2001; Martoriati *et al*, 2005).

The gene encoding *BECN1*, which is required for canonical autophagy (Liang *et al*, 1999; Codogno *et al*, 2012), maps to a tumor susceptibility locus on chromosome 17q21 that is monoallelically deleted in a high proportion of sporadic breast and ovarian carcinomas (Aita *et al*, 1999). Moreover, the expression levels of *BECN1* are reduced in a panel of human neoplasms, including brain tumors as well as gastric and colorectal carcinomas (Rubinshtein *et al*, 2012). Often, *BECN1* is co-deleted with breast cancer 1, early onset (*BRCA1*), a well-known oncosuppressor gene whose loss is associated with familial forms of breast and ovarian carcinoma (Futreal *et al*, 1994). This has recently led some investigators to propose that the loss of *BECN1* does not etiologically contribute to oncogenesis (Laddha *et al*, 2014). However, in two large, independent cohorts of breast carcinoma patients, reduced levels of *BECN1*-coding, but not *BRCA1*-coding, mRNA have been associated with several adverse prognostic features, including estrogen receptor negativity, *HER2* overexpression, basal phenotype, *TP53* mutations, and advanced tumor grade, as well as with decreased patient survival (Tang *et al*, 2015).

Further corroborating the notion that *BECN1* constitutes a haploinsufficient oncosuppressor protein (Yue *et al*, 2003), various physical and functional activators of *BECN1* have been ascribed with tumor-suppressive functions. The expression of *DAPK1*, *smARF* and several BH3-only proteins is lost or reduced as a result of genetic or epigenetic alterations in human tumors of distinct histological origin (Faderl *et al*, 1999; Christoph *et al*, 2007). Along similar lines, *AMBRA1* is mutated in a subset of endometrial, colorectal and urinary tract neoplasms (Cianfanelli *et al*, 2015), the gene coding for the *BECN1* activator UV radiation resistance associated (*UVRAG*) is monoallelically mutated in a high proportion of gastric and colonic tumors (Liang *et al*, 2006; Kim *et al*, 2008), and the expression of SH3-domain GRB2-like endophilin B1 (*SH3GLB1*, a positive modulator of *UVRAG* best known as *BIF1*) (Takahashi *et al*, 2007), is reduced in colorectal carcinomas (Takahashi *et al*, 2007; Coppola *et al*, 2008). Thus, the *BECN1* system appears to be negatively regulated in the course of several paradigms of malignant transformation. As an exception to this trend, the *Becn1*<sup>+/-</sup> genotype delays, rather than accelerates, lymphoid oncogenesis in mice lacking the *ATM* serine/threonine kinase (Valentin-Vega *et al*, 2012). Perhaps, such an oncogenic function of *BECN1* reflects the accrued dependency of *Atm*<sup>-/-</sup> cells on mitochondrial respiration and role of autophagy in the maintenance of a functional mitochondrial network. Of note, *BECN1* and several of its interactors, including *UVRAG* and *VPS34*, are also involved in the late steps of endocytosis (McKnight *et al*, 2014). Moreover, *UVRAG* contributes to genomic stability in an autophagy-unrelated manner (Zhao *et al*, 2012). Finally, the oncosuppressive functions of *AMBRA1* appear to stem, at least in part, from its ability to promote the dephosphorylation-dependent degradation of *MYC* (Cianfanelli *et al*, 2015). Thus, the impact of the *BECN1* system on oncogenesis and tumor progression may be independent of autophagy, at least in some settings.

Germline mutations in *RB1* cause a heritable form of retinoblastoma, a rapidly developing cancer that develops from immature retinal cells (Friend *et al*, 1986). Sporadic *RB1* mutations have been detected in a wide spectrum of neoplasms including osteosarcomas, small cell lung carcinomas and breast carcinomas (Cryns *et al*, 1994). *RB1* is well known for its ability to inhibit various members of the E2F transcription factor family, hence preventing cell cycle progression in the absence of growth factors. The actual role of E2F proteins in autophagic responses, however, remains unclear. On the one hand, the inhibition of E2F by *RB1* has also been linked to the activation of autophagy (Jiang *et al*, 2010), perhaps as a result of declining levels of *BCL2* and consequent *BECN1* derepression (Gomez-Manzano *et al*, 2001). On the other hand, E2F1 has been shown to promote the synthesis of various components of the autophagic machinery, including *ATG1*, *ATG5* and *LC3* (Polager *et al*, 2008). Thus, the ability of E2F family members to regulate autophagy may exhibit some degree of context-dependency.

A panel of genetic conditions characterized by an increased risk of breast, thyroid and endometrial tumors cumulatively referred to as PTEN hamartoma tumor syndrome (PHTS) is provoked by germline mutations in phosphatase and tensin homolog (*PTEN*), an enzyme that promotes autophagy by functionally antagonizing *PI3K* signaling (Liaw *et al*, 1997; Marsh *et al*, 1997; Arico *et al*, 2001). Moreover, the inactivation of *PTEN* as a result of sporadic mutations is very common among human cancers (Sansal & Sellers, 2004). *DIRAS* family, GTP-binding RAS-like 3 (*DIRAS3*), another functional antagonist of *PI3K* with pro-autophagic activity (Lu *et al*, 2008), is frequently underexpressed in breast and ovarian carcinoma upon loss of heterozygosity or promoter hypermethylation (Yu *et al*, 1999; Feng *et al*, 2008).

Germline mutations in neurofibromin 1 (*NF1*), serine threonine kinase 11 (*STK11*), *TSC1* and *TSC2* are also associated with syndromes characterized by an increased incidence of both benign and malignant neoplasms, notably, type I neurofibromatosis (*NF1*), Peutz–Jeghers's syndrome (*STK11*) and tuberous sclerosis (*TSC1* and *TSC2*) (Fountain *et al*, 1989; van Sleghenhorst *et al*, 1997; Hemminki *et al*, 1998). Moreover, somatic mutations in *STK11* have been documented in a considerable percentage of squamous cell carcinomas and lung adenocarcinomas (Ji *et al*, 2007). As a common denominator, the inactivation of *NF1* (an upstream inhibitor of *RAS*), *STK11* (an activator of *AMPK* best known as *LKB1*), *TSC1* or *TSC2* promotes *MTORC1* signaling, hence stimulating cell proliferation and inhibiting autophagic responses (Xu *et al*, 1990; Inoki *et al*, 2002; Shaw *et al*, 2004; Johannessen *et al*, 2005). In spite of such an *MTORC1*-dependent inhibition of autophagy, the monoallelic loss of *Becn1* suppresses spontaneous renal tumorigenesis in *Tsc2*<sup>+/-</sup> mice (Parkhitko *et al*, 2011). This may indicate that the *Tsc2*<sup>+/-</sup> genotype does not completely block autophagic responses, or that—at least in this model—*BECN1* exerts oncosuppressive functions that are mainly autophagy-independent. The deletion of *Rb1cc1* has also been shown to suppress, rather than enhance, mammary tumorigenesis driven by the polyoma middle T antigen (PyMT) (Hara *et al*, 2008; Wei *et al*, 2011). Although in this setting the absence of *FIP200* was indeed associated with autophagic defects in PyMT-expressing malignant cells, such alterations were not etiologically linked to oncogenesis. This is intriguing as *FIP200* (which is also involved in the regulation of *RB1*) is considered as a classical oncosuppressor protein and is lost as a consequence of

large deletions in a high fraction of breast carcinomas (Chano *et al*, 2002).

The von Hippel–Lindau syndrome, an inherited disorder characterized by the formation of tumors and cysts at various anatomical locations, is provoked by germline mutations in von Hippel–Lindau tumor suppressor, E3 ubiquitin protein ligase (*VHL*) (Seizinger *et al*, 1988). Moreover, *VHL* is affected by somatic mutations in a large proportion of renal cell carcinomas (Shuin *et al*, 1994). In *VHL*-deficient cells, hypoxia-inducible factor 1 (*HIF-1*) accumulates irrespective of oxygen concentration (because *VHL* normally targets it to proteasomal degradation), resulting in the transactivation of genes involved in bioenergetic metabolism and angiogenesis (Maxwell *et al*, 1999). *VHL* suppresses autophagy by an epigenetic mechanism involving *miR-204* (Mikhaylova *et al*, 2012; Hall *et al*, 2014). *MiR-204* targets *LC3B* as well as transient receptor potential cation channel, subfamily M, member 3 (*TRPM3*), a  $Ca^{2+}$ - and  $Zn^{2+}$ -conductive channel that potently stimulates autophagy. Moreover, the hyperactivation of *HIF-1* supports mitophagy upon the upregulation of *BCL2*/adenovirus E1B 19 kDa interacting protein 3 (*BNIP3*) and *BNIP3*-like (*BNIP3L*) even in normoxic conditions (Tracy *et al*, 2007; Zhang *et al*, 2008; Bellot *et al*, 2009; Wilkinson *et al*, 2009). Thus, the loss of *VHL* may sustain tumor progression not only as it promotes angiogenesis, but also as it stimulates autophagy. Of note, *HIF-1* is not a *bona fide* oncoprotein, yet multiple cancer cells express increased *HIF-1* levels and rely on *HIF-1* for survival (Luo *et al*, 2009).

The transcription factor forkhead box O1 (*FOXO1*) is mutated in a percentage of diffuse large B-cell lymphoma (DLBCL) cases and is etiologically involved in at least one model of murine oncogenesis (Huang *et al*, 2005). *FOXO1* is classically ascribed with oncosuppressive functions by virtue of its multipronged pro-apoptotic activity (Fu & Tindall, 2008). In addition, a cytosolic pool of *FOXO1* seems to be required for stress-induced autophagy as a consequence of its physical interaction with *ATG7* (Zhao *et al*, 2010). Along similar lines, *ATG5* is expressed at low levels in primary melanomas as compared to benign nevi, resulting in reduced autophagic proficiency (Liu *et al*, 2013a), and its absence favors oncogenesis in mice (Takamura *et al*, 2011). Although neither *FOXO1* nor *ATG5* can be considered as *bona fide* oncosuppressor proteins, these results support the contention that various factors with oncosuppressive functions promote autophagic responses.

Taken together, the observations presented above indicate that the hyperactivation of oncoproteins, as well the inactivation of oncosuppressor proteins, most often limits autophagic responses and that the partial suppression of autophagy underlies several (though perhaps not all) paradigms of oncogenesis. The actual impact of primary oncogenic events on autophagy, however, may be influenced (at least to some extent) by the presence/absence of additional genetic defects, and hence is likely to evolve along with tumor progression.

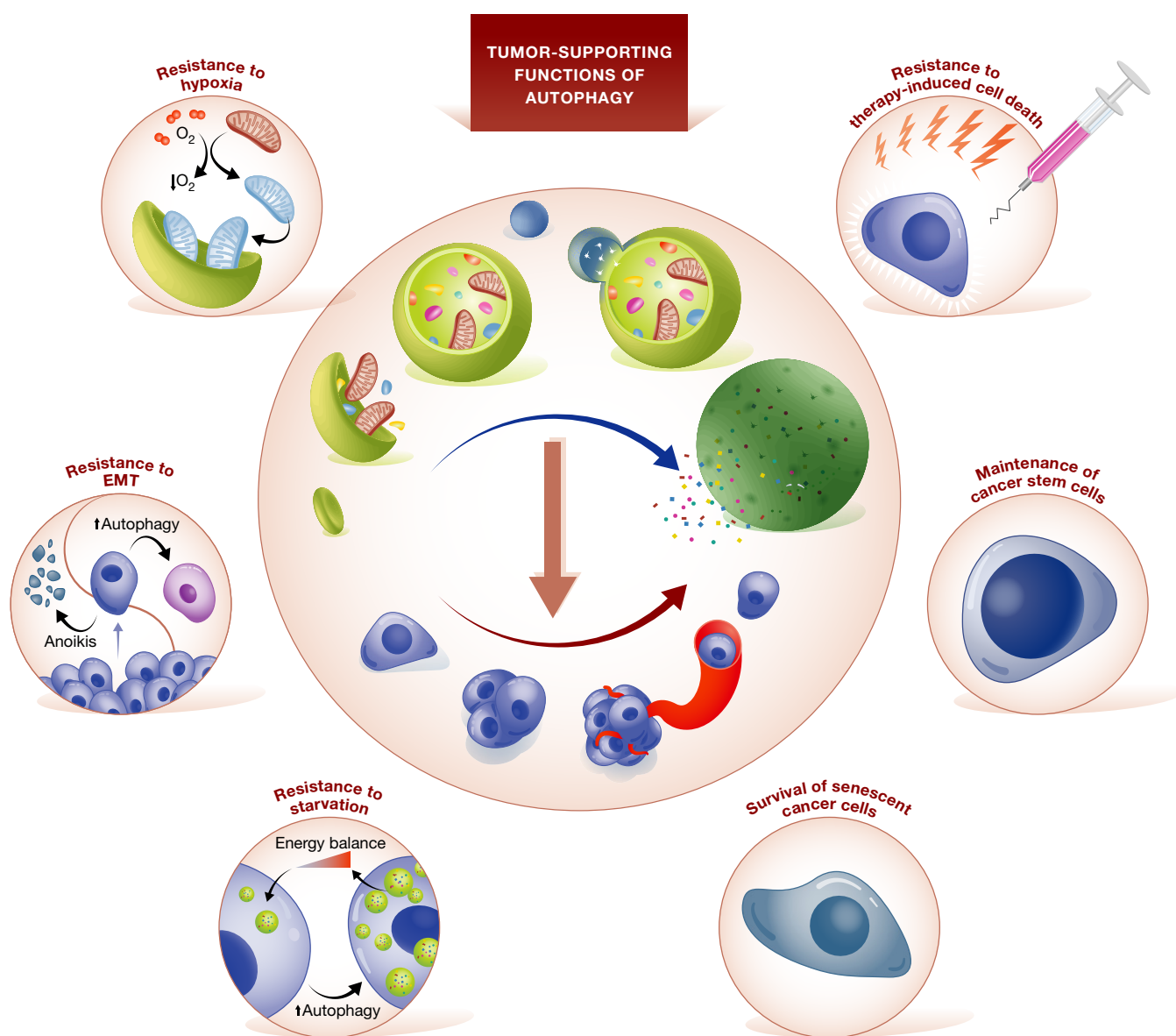
## Autophagy and tumor progression

Autophagic responses generally support the growth and progression of established tumors by reducing their sensitivity to cell-intrinsic as well as microenvironmental stimuli that would normally promote their demise, in particular upon the so-called epithelial-to-mesenchymal transition (Kroemer *et al*, 2010; Avivar-Valderas *et al*,

2013; Cai *et al.*, 2014). This notion is supported by a growing amount of data indicating that defects in the autophagic machinery often restrain the proliferation, dissemination and metastatic potential of malignant cells, as discussed below. Moreover, advanced human tumors generally exhibit an increased autophagic flux, correlating with an invasive/metastatic phenotype, high nuclear grade, and poor disease outcome (Lazova *et al.*, 2012; Mikhaylova *et al.*, 2012).

Although mice with a systemic mosaic deletion of *Atg5* or a liver-specific knockout of *Atg7* develop spontaneous hepatic neoplasms more frequently than their wild-type counterparts (see above), these

malignancies are mostly benign (pointing to defects in tumor progression) and their size can be further decreased by the simultaneous deletion of *Sqstm1* (Takamura *et al.*, 2011). In accord with these data, p62 has been shown to support the progression of both endogenous (ERBB2-driven) and xenografted mammary tumors through a variety of mechanisms, including the activation of NRF2 (see above) (Chen *et al.*, 2013; Cai-McRae *et al.*, 2014). As opposed to their autophagy-competent counterparts, highly metastatic hepatocellular carcinoma cell lines infected with lentiviruses that stably downregulate BECN1 or ATG5 are virtually unable to survive within the metastatic niche, although they normally proliferate, invade



**Figure 3. Tumor-supporting functions of autophagy.**

Once malignant transformation has occurred, autophagy is believed to promote tumor progression and resistance to therapy. Such tumor-supporting functions reflects the ability of autophagy to: (1) improve the resistance of cancer cells to endogenous conditions that normally provoke cell death, such as the detachment from the basal membrane, hypoxia and nutrient deprivation; (2) render transformed cells less sensitive to therapy-induced cell death; (3) sustain the survival of cancer cells that enter a state of dormancy or senescence in response to therapy; and (4) ensure the maintenance of the cancer stem cell compartment. EMT, epithelial-to-mesenchymal transition.

surrounding tissues and undergo the epithelial-to-mesenchymal transition (Peng *et al*, 2013). Along similar lines, the shRNA-mediated downregulation of Atg5 or p62 abolishes the ability of *Tsc2*<sup>-/-</sup> *Trp53*<sup>-/-</sup> MEFs to develop macroscopic tumors upon inoculation into nude mice (Parkhitko *et al*, 2011). Moreover, the robust anti-metastatic effects of N-myc downstream regulated 1 (NDRG1) have recently been ascribed to its ability to suppress stress-induced autophagic responses (Sahni *et al*, 2014).

The lung-specific deletion of *Atg7* during the late stages of *BRAF*<sup>V600E</sup>-driven carcinogenesis favors the development of small oncocytomas (which are relatively benign tumors) rather than adenocarcinomas, a shift that is accompanied by the accumulation of dysfunctional mitochondria and an increased dependency on exogenous glutamine (Strohecker *et al*, 2013). Similar results have been obtained in models of *KRAS*<sup>G12D</sup>-driven lung and pancreatic carcinogenesis, upon the tissue-specific deletion of *Atg5* or *Atg7* (Guo *et al*, 2013a; Rosenfeldt *et al*, 2013; Rao *et al*, 2014; Yang *et al*, 2014), as well as in models of breast carcinoma driven by the mammary-gland specific knockout of partner and localizer of *BRCA2* (*Palb2*), upon the monoallelic deletion of *Becn1* (Huo *et al*, 2013). Intriguingly, the downregulation of *Atg1* (the fly ortholog of ULK1) by RNA interference also suppressed a hyperproliferative eye phenotype caused in *Drosophila melanogaster* by the ectopic expression of *Ras*<sup>L2V</sup> (a constitutively active variant of the fly ortholog of mammalian RAS proteins) but had an opposite effect on the overgrowth of the eye epithelium provoked by mutations in the oncosuppressor gene scribbled (*scrib*) (Perez *et al*, 2014). Similarly, the individual depletion of 12 distinct components of the autophagic machinery (i.e., *Atg1*, *Atg6*, *Atg12*, *Atg5*, *Atg7*, *Atg4a*, *Atg4b*, *Atg8a*, *Atg8b*, *Atg3*, *Atg9* and *Atg18*) promoted, rather than limited, the overgrowth of adult *Drosophila* eyes and their larval precursor tissues in so-called *eyeful* flies (a model of Notch-driven carcinogenesis) (Perez *et al*, 2014).

In some models of endogenous mammalian carcinogenesis, the *Trp53*<sup>-/-</sup> genotype prevents genetic interventions that target the autophagic machinery from provoking metabolic and bioenergetic alterations that limit tumor progression (Huo *et al*, 2013; Rosenfeldt *et al*, 2013; Rao *et al*, 2014). Conversely, the response of pancreatic cancer cells, xenografts and *KRAS*<sup>G12D</sup>-driven autochthonous adenocarcinomas to genetic or pharmacological autophagy inhibition persists in the context of TP53 loss-of-heterozygosity (Yang *et al*, 2011; Yang *et al*, 2014).

*KRAS*<sup>G12D</sup>-driven pancreatic adenocarcinoma cells entering a state of dormancy (rather than succumbing) in response to oncogene ablation (i.e., the shutdown of oncogenic *KRAS* signaling) have recently been shown to activate autophagy to efficiently counteract metabolic stress (Viale *et al*, 2014). Of note, whereas primary *KRAS*<sup>G12D</sup>-expressing cells generally exhibit increased glucose and glutamine uptake, as well as an elevated anabolic flux via the pentose phosphate pathway (Ying *et al*, 2012; Rosenfeldt *et al*, 2013), the survival of oncogene-depleted pancreatic adenocarcinoma cells critically relies on oxidative phosphorylation (Viale *et al*, 2014). Thus, exposing such dormant pancreatic adenocarcinoma cells to the inhibitor of oxidative phosphorylation oligomycin reportedly abolishes their ability to form tumors upon *KRAS*<sup>G12D</sup> re-expression (Viale *et al*, 2014).

Interestingly, *KRAS*<sup>G12D</sup>-expressing pancreatic adenocarcinoma cells driven into dormancy upon oncogene ablation also display

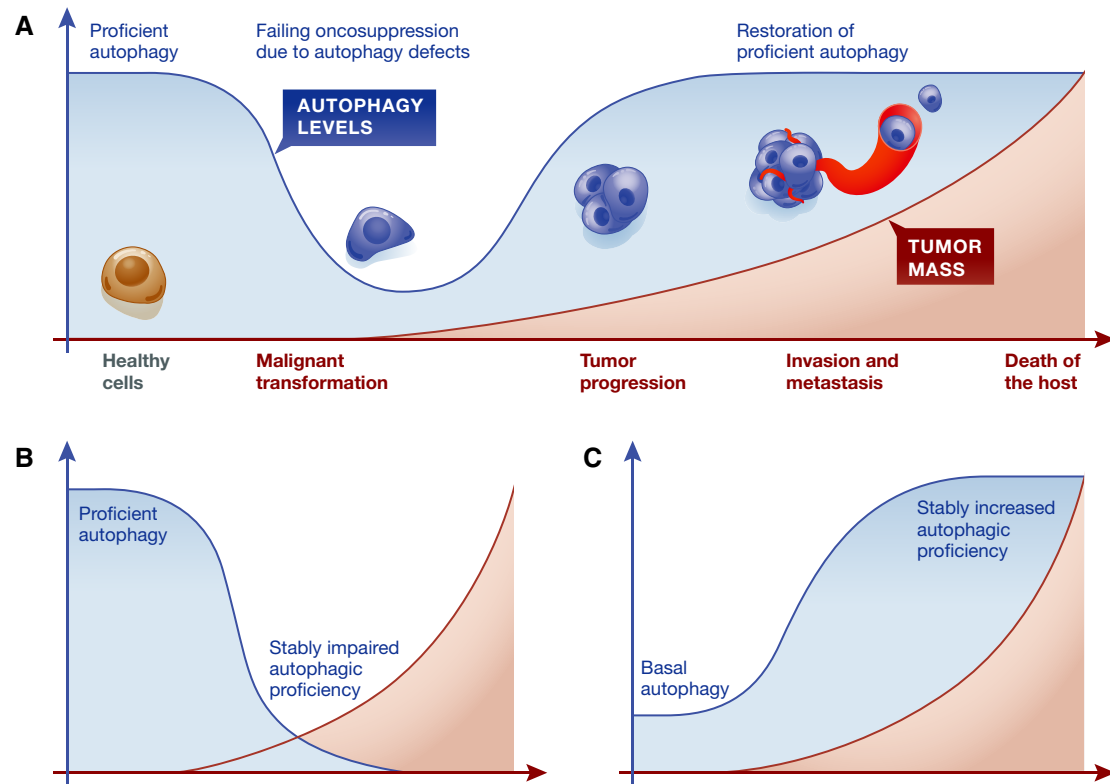
functional and phenotypic features of cancer stem cells (CSCs) (Viale *et al*, 2014). Moreover, mammary CSCs (which propagate in culture as mammospheres) are often characterized by an elevated autophagic flux, and their ability to efficiently form tumors *in vivo* appears to rely on autophagy, as tumor formation can be abolished by the genetic inhibition of *BECN1* or *ATG4A* (Gong *et al*, 2013; Wolf *et al*, 2013). Thus, autophagy may also sustain tumor progression by preserving the viability of the CSC compartment and/or by promoting the persistence of dormant cancer cells (Viale *et al*, 2014).

Cancer cells isolated from established tumors and subjected to the genetic or pharmacological inhibition of autophagy are less resistant to exogenous stimuli than their wild-type counterparts (Boya *et al*, 2005; Amaravadi *et al*, 2007; Kroemer *et al*, 2010). In line with this notion, autophagy-deficient tumors are often more sensitive to several chemotherapeutic agents as well as to radiation therapy than their autophagy-proficient counterparts (Janku *et al*, 2011; Ko *et al*, 2014; Levy *et al*, 2014). This does not necessarily hold true for immunocompetent mice. Indeed, autophagic responses preceding the demise of cancer cells exposed to a selected panel of agents are required (though not sufficient) for cell death to be perceived as immunogenic and hence to elicit a therapeutically relevant immune response (Kroemer *et al*, 2013; Ko *et al*, 2014). Cancer cells exposed to therapeutic interventions can also undergo senescence (Lopez-Otin *et al*, 2013). Although senescent cells do not proliferate, they may support disease relapse by releasing a wide panel of pro-inflammatory and mitogenic cytokines into the micro-environment (underlying the so-called senescence-associated secretory phenotype, SASP) (Lopez-Otin *et al*, 2013). Interestingly, these cells are highly dependent on autophagic responses for survival, and pharmacological inhibitors of autophagy have been shown to synergize with various chemotherapeutics in experimental models of lymphoma that are susceptible to acquire the SASP in response to treatment (Young *et al*, 2009; Dorr *et al*, 2013).

Taken together, these observations suggest that autophagy supports the progression of established neoplasms through several mechanisms (Fig 3) and that pharmacological inhibitors of autophagy may exert robust antineoplastic effects, at least in some settings.

## Concluding remarks

Based on the data presented above, it is tempting to speculate that the multistep process leading from a healthy tissue to a metastatic and therapy-resistant, and hence life-threatening, neoplasm involves a temporary loss of autophagic competence (or the gain of molecular functions that antagonize, at least transiently, autophagy-dependent oncosuppression). Initially, defects in the autophagic process might facilitate the acquisition of malignant features by healthy cells. Later on, once malignancy is established, the restoration of proficient autophagic responses may be essential to support the survival, proliferation and growth of cancer cells in the presence of adverse microenvironmental conditions (Fig 4A). How proficient autophagic responses are reconstituted after an initial phase of autophagy inhibition, however, has not yet been established. As a possibility, the genetic or epigenetic instability that characterizes progressing tumors may restore autophagy in specific cells,



**Figure 4. Autophagy in malignant transformation and tumor progression.**

(A) Healthy cells appear to be protected from malignant transformation by proficient autophagic responses. Conversely, autophagy promotes tumor progression and therapy resistance in a variety of models. Thus, the transition of a healthy cell toward a metastatic and therapy-insensitive neoplasm may involve a temporary (but not a stable) loss in autophagy competence. The mechanisms underlying the restoration of proficient autophagic responses after malignant transformation remain to be elucidated. (B, C) In specific settings, oncogenesis and tumor progression may rely on a permanent loss (B) or gain (C) of autophagic proficiency.

rendering them able to overcome their neighboring autophagy-incompetent counterparts. Formal experimental evidence in support of this model is lacking. At least in some settings, oncogenesis and tumor progression may indeed rely on a stable loss or gain of autophagic proficiency (Fig 4B and C).

Importantly, genetic data indicating that the inhibition of autophagy exerts *bona fide* antineoplastic effects against established tumors have been obtained mainly in RAS-driven or RAS-related (*BRAF*<sup>V600E</sup>-driven) models of oncogenesis (Guo *et al*, 2013a; Rosenfeldt *et al*, 2013; Strohecker *et al*, 2013; Perez *et al*, 2014; Rao *et al*, 2014; Yang *et al*, 2014). In other scenarios, including the loss of *scrib* and the *eyeful* genotype in *Drosophila*, disabling autophagy by genetic means *de facto* accelerates tumor progression (Perez *et al*, 2014). Moreover, the antineoplastic effects of genetic and pharmacological interventions that inhibit autophagy vary in models in which the TP53 system is lost by different modalities (i.e., homozygous knockout versus loss-of-heterozygosity) (Yang *et al*, 2011; Huo *et al*, 2013; Rosenfeldt *et al*, 2013; Rao *et al*, 2014; Yang *et al*, 2014). Thus, the impact of autophagy on tumor progression may exhibit a significant degree of context dependency. Accordingly, recent data indicate that only tumors that are addicted to autophagy even in nutrient-rich conditions and in the absence of stressful stimuli respond to autophagy inhibitors *in vivo* (Maycotte *et al*, 2014). This suggests that only a fraction of cancer patients may benefit from the administration of autophagy inhibitors. Along

similar lines, autophagy has been shown to underlie, at least in part, the therapeutic activity of some anticancer regimens (Salazar *et al*, 2009; Torres *et al*, 2011; Vara *et al*, 2011). Moreover, autophagy is required not only for the emission of immunostimulatory signals by malignant cells succumbing to specific anticancer agents (Kroemer *et al*, 2013), but also for the activation of tumor-targeting innate and adaptive immune responses (Ma *et al*, 2013). Efforts should therefore be focused on the identification of precise clinical scenarios in which autophagy supports, rather than counteracts, disease progression and resistance to therapy. This is particularly important not only because autophagy inhibitors may one day become part of the clinical routine, but also because most (if not all) anticancer agents that are currently employed in the clinic modulate autophagy (Kroemer *et al*, 2010).

Of note, the genetic inhibition of autophagy in models of mammalian carcinogenesis has near-to-invariably been achieved with the whole-body or conditional (heterozygous or homozygous) knockout of *Atg5*, *Atg7* or *Becn1* (Guo *et al*, 2013a; Rosenfeldt *et al*, 2013; Strohecker *et al*, 2013; Rao *et al*, 2014; Yang *et al*, 2014). As an increasing number of activities is being ascribed to these and other autophagy mediators (Cosse *et al*, 2010; Lee *et al*, 2012; Liu *et al*, 2012a; Moscat & Diaz-Meco, 2012; Zhao *et al*, 2012; Maskey *et al*, 2013; Elgendy *et al*, 2014), it remains possible that other, autophagy-independent functions of ATG5, ATG7 and BECN1 support the progression of *KRAS*<sup>G12D</sup>- and *BRAF*<sup>V600E</sup>-driven tumors.



In experimental settings, the pharmacological inhibition of autophagy is most often realized with the administration of chloroquine (CQ) and hydroxychloroquine (HCQ), two lysosomotropic drugs approved by the US Food and Drug Administration for the prophylactic treatment of malaria and for the management of (chronic, discoid or systemic) lupus erythematosus as well as acute or chronic rheumatoid arthritis (Rubinsztein *et al*, 2012). Similar to other lysosomotropic agents, both CQ and HCQ block autophagy by inhibiting the fusion between autophagosomes with lysosomes and their degradation (Rote & Rechsteiner, 1983). Results from several Phase I–II clinical data indicate that HCQ can be safely employed at relatively high doses to improve the clinical activity of radiation therapy as well as of distinct anticancer chemotherapeutics, including temozolomide (an alkylating agent), vorinostat (a histone deacetylase inhibitor) and bortezomib (a proteasome inhibitor) (Barnard *et al*, 2014; Mahalingam *et al*, 2014; Rangwala *et al*, 2014a; Rangwala *et al*, 2014b; Rosenfeld *et al*, 2014; Vogl *et al*, 2014; Wolpin *et al*, 2014). However, the potency and specificity of HCQ and CQ are poor, and both these compounds have been shown to mediate antineoplastic effects via multiple autophagy-independent pathways, including lethal lysosomal destabilization (Boya *et al*, 2003;

Maycotte *et al*, 2012) and the normalization of the tumor vasculature (Maes *et al*, 2014). Thus, the abundant scientific literature concluding that pharmacological inhibitors of autophagy constitute a convenient means to arrest tumor progression or sensitize malignant cells to therapy based on results obtained with CQ and HCQ only should be taken with caution. Lys05, a potent dimeric variant of CQ, is currently being characterized in preclinical tumor models (McAfee *et al*, 2012). Lys05, however, seems to share the limited specificity of CQ and HCQ. Small molecules that block autophagy in a highly specific manner are therefore urgently awaited. Recently, a specific VPS34 inhibitor has been developed and shown to efficiently inhibit autophagy. However, its putative antineoplastic effects may reflect the pleiotropic activity of VPS34, which is also involved in non-autophagic vesicle trafficking (Ronan *et al*, 2014). Finally, it will be interesting to develop molecules that inhibit autophagy in malignant cells but not in their normal counterparts, perhaps by targeting upstream signal transducers rather than downstream effectors. Proof-of-principle data in support of the therapeutic activity of such an approach in preclinical models have already been generated (Wilkinson *et al*, 2009). This is particularly relevant given the key contribution of autophagy to the maintenance of homeostasis in healthy cells. Indeed, at least on theoretical grounds, efficiently inhibiting autophagy in non-transformed cells may have deleterious consequences ranging from an accrued propensity to malignant transformation to overt cytotoxicity.

Intriguingly, several experimental maneuvers that increase the lifespan of model organisms as evolutionary distant as nematodes, flies and mice, including caloric restriction as well as the administration of the mTOR inhibitor rapamycin, activate autophagy (Harrison *et al*, 2009; Morselli *et al*, 2010; Madeo *et al*, 2014). Moreover, these interventions generally lose their lifespan-extending activity in autophagy-deficient hosts (Morselli *et al*, 2010; Madeo *et al*, 2014). However, to which extent the lifespan-prolonging effects of autophagy directly relate to its major oncosuppressive functions remains to be determined.

Recently, several studies have demonstrated that autophagy is regulated by epigenetic alterations, including histone methylation and acetylation (Artal-Martinez de Narvajás *et al*, 2013; Lam *et al*, 2013; Eisenberg *et al*, 2014). In addition, transcription factors other than TP53 and HIF-1, such as transcription factor EB (TFEB) and cAMP-responsive element binding protein 1 (CREB1), are intimately involved in autophagic responses (Settembre *et al*, 2011; Seok *et al*, 2014). The precise mechanisms through which cancer-associated epigenetic alterations (and/or the consequent transcriptional reprogramming) modulate autophagy have not yet been elucidated. Obtaining profound insights into this issue may pave the way to the development of novel, cancer-specific inhibitors of autophagy with therapeutic potential.

Irrespective of these incognita, autophagy stands out as key system for the maintenance of homeostasis, hence exerting a differential impact on malignant transformation and tumor progression (Box 1).

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### Box 1: Reconciling the effects of autophagy on malignant transformation and tumor progression

- Autophagy is a crucial mechanism for the maintenance of intracellular homeostasis, in baseline conditions as well as in response to stress.
- Autophagy has been attributed tumor-suppressive as well as tumor-promoting functions, raising doubts on the actual therapeutic value of autophagy inhibitors for cancer therapy.
- According to a growing literature, autophagy inhibits malignant transformation, that is, the conversion of a completely normal cell into a cell that can potentially form a tumor, and is required for efficient anticancer immunosurveillance.
- Conversely, autophagy often supports tumor progression, that is, the process whereby a (pre)-neoplastic cell acquires the ability to grow unrestrained locally, promote angiogenesis, undergo the epithelial-to-mesenchymal transition, reach a metastatic niche through circulation and form secondary lesions, as well as resistance to various forms of anticancer therapy.
- From a cell-centered perspective, autophagic responses near-to-invariably exert beneficial effects, but these are detrimental for the host when they occur in cells that have already initiated malignant transformation.
- In line with this notion, pharmacological inhibitors of autophagy exert antineoplastic effects against established tumors, especially in combination with other forms of therapy.
- However, highly targeted inhibitors of autophagy for use in humans are not available, and the molecules employed so far to this aim (i.e., chloroquine and hydroxychloroquine) have several, therapeutically relevant off-target effects.
- Moreover, the true impact of autophagy on oncogenesis and tumor progression may exhibit a significant degree of context dependency.
- Thus, it is critical to characterize specific neoplasms for their actual dependency on autophagy before implementing the use of autophagy inhibitors in the clinical routine.

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### Author contributions

LG, KMR and GK conceived the review. LG wrote the review, centralized and integrated comments from co-authors, conceived the figures, and revised the review upon editorial feedback. JMBS designed the figures. FP constructed the table. All authors corrected the review and provided valuable input to obtain a consensus view.

### Conflict of interest

Vassiliki Karantza is an employee of Merck Research Laboratories. All other authors declare that they have no conflict of interest.

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