Chapter 3
Autophagy and Tumor Cell Metabolism

Ahmed Hamaï, Joelle Botti, Maryam Mehrpour, and Patrice Codogno

Contents

A. Hamaï • M. Mehrpour
INSERM U1151, CNRS UMR 8253, Institut Necker Enfants-Malades, Université Paris 5 René Descartes, 14, rue Maria Helena Vieira Da Silva CS61431, 75993 Paris cedex 14, France
e-mail: ahmed.hamai@inserm.fr; maryam.mehrpour@inserm.fr

J. Botti
INSERM U1151, CNRS UMR 8253, Institut Necker Enfants-Malades, Université Paris 5 René Descartes, 14, rue Maria Helena Vieira Da Silva CS61431, 75993 Paris cedex 14, France

Lariboisière-Saint Louis, Université Paris 7 Denis Diderot, 75010 Paris, France
e-mail: joelle.botti@inserm.fr

P. Codogno (✉)
INSERM U1151, CNRS UMR 8253, Institut Necker Enfants-Malades, Université Paris 5 René Descartes, 14, rue Maria Helena Vieira Da Silva CS61431, 75993 Paris cedex 14, France
e-mail: patrice.codogno@inserm.fr

S. Mazurek, M. Shoshan (eds.), *Tumor Cell Metabolism*,
DOI 10.1007/978-3-7091-1824-5_3

3.1 Introduction

The word autophagy, from the Greek for *self-eating*, refers to the catabolic processes through which the cell recycles its own constituents in the lysosome (Mizushima et al. 2008; Yang and Klionsky 2010). This chapter will focus on macroautophagy (hereafter referred to as autophagy), because the evidence that the other forms of autophagy play any role in tumor biology is relatively limited.

Autophagy starts with the formation of a double-membrane bound vacuole, known as the autophagosome, that engulfs fractions of the cytoplasm in either an unselective or a selective manner via the activity of the autophagy adaptors (SQSTM1/p62, NBR1, NDP52, and optineurin) that form a bridge between the target and the growing autophagosome membrane (Mizushima and Komatsu 2011; Boya et al. 2013). After being formed, most autophagosomes receive input from the endocytic vesicles to form an amphisome before the autophagic cargo undergoes complete degradation in the lysosomal lumen (Fig. 3.1). Basal rate autophagy exercises quality control on the cytoplasm of most cells by removing damaged organelles and protein aggregates. Autophagy is a response to a range of stimuli and in most cases protects cells against stressful situations (Kroemer et al. 2010). In response to starvation, autophagy is important for the lysosomal recycling of metabolites into the cytoplasm, where they are reused either as a source of energy or to provide building blocks for the synthesis of new macromolecules.

The discovery of *ATG* (autophagy-related) genes in eukaryotic cells and that of the role of ATG proteins in the formation of autophagosomes were milestones in the understanding of the molecular aspects of autophagy (Mizushima et al. 2011). ATG proteins are recruited on a membrane known as the phagophore. Several cellular pools of membranes contribute to the formation of the phagophore (Hamasaki et al. 2013; Moreau et al. 2013). The hierarchical intervention of ATG with other proteins leads to the elongation and the closure of the membrane to form the autophagosome (Fig. 3.1). At a molecular level, the first step in the initiation of autophagy is the activation of a molecular complex containing the serine/threonine kinase ULK1 (the mammalian ortholog of Atg1 in yeast). The activation of this complex is downregulated by MTORC1, which integrates multiple signaling pathways that are sensitive to the availability of amino acids, ATP, growth factors, and the level of ROS. The expansion, curvation, and closure of the autophagosome are controlled by another molecular complex including phosphatidylinositol 3-kinase (PI3K) and Beclin 1 (the mammalian ortholog of Atg6 in yeast), which allows the production of phosphatidylinositol 3-phosphate (PI3P) to occur, and the subsequent recruitment of PI3P-binding proteins WIPI1/2 and two ubiquitin-like conjugation systems ATG12–ATG5-ATG16L and LC3–PE. The final fusion with lysosome requires small Rab GTPases and the transmembrane protein LAMP2. Acid hydrolases and the cathepsins present in the lysosomal lumen degrade the autophagosomal cargoes.

Advances in our understanding of the autophagic process paved the way for the discovery of the importance of autophagy in development, tissue homeostasis,

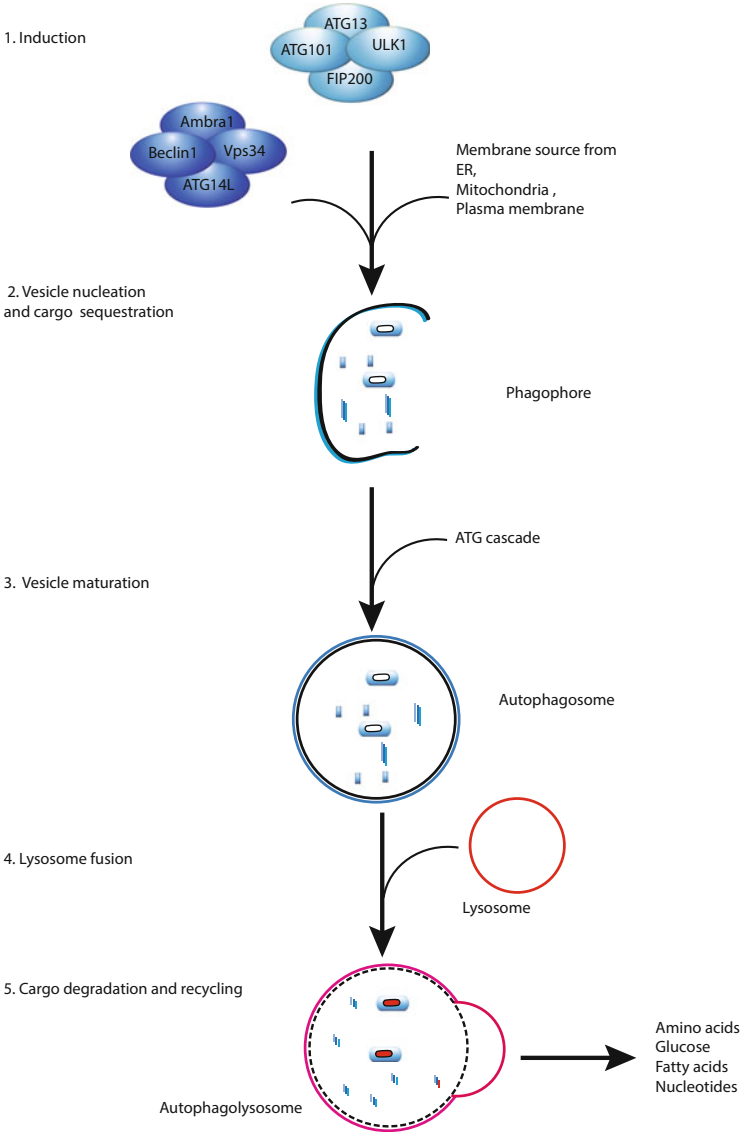

Fig. 3.1 Overview of the autophagy pathway**.** Autophagy is orchestrated by the coordinated action of Atg proteins to form the autophagosome from the pre-autophagosomal structure to which Atg proteins are hierarchically recruited to form the isolation membrane of the phagophore. Through a process of maturation and fusion, these membrane-bound structures become autolysosomes, degrading their contents and releasing amino acids, fatty acids, nucleotides, and other molecules required to maintain cell metabolism

metabolism, the immune response, and various diseases (Deretic and Levine 2009; Ravikumar et al. 2010; Rubinsztein et al. 2012; Choi et al. 2013). Interest in the role of autophagy in cancer stems from the discovery that *BECN1* (the gene that encodes Beclin 1, the human ortholog of the yeast Atg6) is a haplo-insufficient tumor suppressor gene (Rubinsztein et al. 2012; Choi et al. 2013). In fact, it appears that autophagy is under the control of a large panel of oncogenes and products of tumor suppressor genes (Botti et al. 2006; Maiuri et al. 2009). However, the role of autophagy in tumors is complex and ranges from a tumor-suppressive role to a role in helping cells to adapt to the environment. In cancer cells, autophagy fulfills a dual role, having both tumor-promoting and tumor-suppressing properties (Liu and Ryan 2012; White 2012; Lorin et al. 2013). By maintaining cellular homeostasis in healthy cells, autophagy prevents DNA damage and genomic instability, which can lead to tumoral transformation. Autophagy can also facilitate oncogene-induced senescence or protect tumors against necrosis and inflammation, thus limiting tumor growth. On the other hand, autophagy can contribute to tumor progression, by allowing tumor cells to survive stressful conditions and sustaining the deep metabolic reorganization that cancer cells undergo after oncogenic transformation. Autophagy also appears to be important in supporting tumor development by maintaining the survival and self-renewal of cancer stem cells (Gong et al. 2013; Guan et al. 2013; Pan et al. 2013).

In this chapter, we will discuss the interplay between autophagy and tumor cell metabolism, the relationship between cell metabolism and the regulation of autophagy by acetylation, and finally the emerging role of autophagy in cancer stem cells.

3.2 Autophagy and Metabolic Adaptation

One of the hallmarks of tumors is the upregulation of cytosolic glycolysis: the conversion of glucose into lactate under hypoxic or normoxic conditions by cancer cells. This "aerobic glycolysis," despite the fact that it reduces efficiency (thus increasing the rate of energy production), is associated with a reduction of the activity of mitochondrial electron chain transport (DeBerardinis 2008). This metabolic reprogramming, also known as the "Warburg effect," is induced by the oncogenic transformation of tumor cells (Fig. 3.2). This metabolic adaptation is associated with cell transformation and seems to require the activation of oncogenes, such as RAS (Dang and Semenza 1999; Manning and Cantley 2007), AKT (Manning and Cantley 2007), and MYC (Gordan et al. 2007), and the inhibition of tumor suppressors, such as p53 (Bensaad et al. 2006; Matoba et al. 2006; Kawauchi et al. 2008). MYC and RAS transformation impair acetyl-CoA production, an essential component of the mitochondrial tricarboxylic acid (TCA) cycle, by blocking its generation from the decarboxylation of pyruvate (White 2012). RAS transformation also impairs acetyl-CoA production by blocking the β-oxidation of fatty acids. In addition, MYC transformation stimulates glycolysis, glutaminolysis,

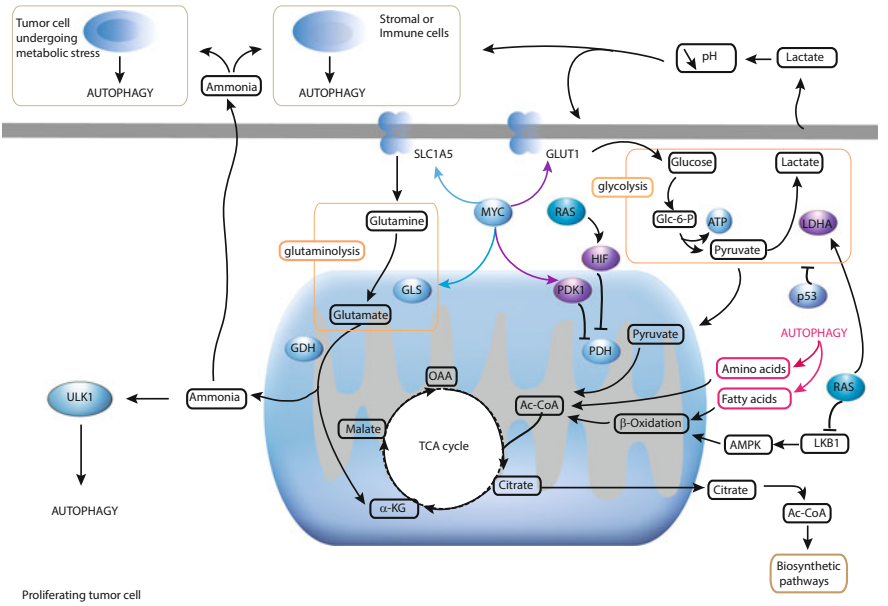

Fig. 3.2 The autophagy and metabolic coupling between tumor and stroma cells**.** In a context of metabolic stress, cancer cells catabolize glutamine to form α-ketoglutarate, which enters the tricarboxylic acid (TCA) cycle—also known as Krebs cycle—and increases mitochondrial activity, thus promoting survival and growth. The activation of oncogenes, such as RAS and MYC, seems to orchestrate the metabolic changes associated with cell transformation. RAS diminishes the pool of acetyl-CoA by three known mechanisms. First, RAS can activate lactate dehydrogenase (LDH), which converts pyruvate to lactate, which is excreted. Second, RAS can activate hypoxia-inducible factor (HIF), thus inhibiting pyruvate dehydrogenase (PDH) and the conversion of pyruvate to acetyl-CoA (Ac-CoA). Third, RAS inhibits liver kinase B1 (LKB1), blocking AMP kinase (AMPK) and β-oxidation. Defective autophagy results in reduced citrate levels, impaired TCA cycle function, and reduced mitochondrial respiration. Autophagy can potentially compensate for the metabolic reprogramming by RAS by degrading proteins and lipids to provide amino acid and fatty acid substrates that produce acetyl-CoA. Tumor cells might also compensate for autophagy impairment by upregulating glycolysis, glutaminolysis, or the reductive carboxylation of α-ketoglutarate (α-KG) from glutamine. The transcription factor Myc favors the "Warburg effect" by increasing the abundance of key glycolytic enzymes, including Glut1, LDHA, and PDK1. Myc stimulates glutamine metabolism by increasing the abundance of glutamine transporters (SLC1A5) and glutaminase (GLS). α-KG is produced via the double deamination of glutamine, a process known as glutaminolysis. Glutamine is first deaminated by GLS to produce glutamate. Glutamate is then converted to α-KG by glutamate dehydrogenase (GDH). Ammonia is generated as a by-product of glutamine deamination and induces autophagy in an autocrine or paracrine fashion through an unknown mechanism, although the kinase ULK1 (unc-51-like kinase 1) seems to be required. Ammonia is a diffusible factor that stimulates the autophagy program in the adjacent stroma cells (in particular, cancer-associated fibroblasts CAFs). Increased aerobic glycolysis occurs in stromal cells, leading to the generation and secretion of high levels of glutamine into the tumor microenvironment, which maintains the tumor cell metabolism. In contrast, ammonia does not interfere with the activity of mTOR, which is a key inhibitor of autophagy. Cancer cells are rendered less sensitive to ammonia by the upregulation of TIGAR, a p53-inducible regulator of glycolysis. The ability of TIGAR to limit autophagy is closely correlated to the suppression of ROS and is p53 independent. Interestingly, the levels of Acetyl-CoA and of NAD^+/NADH, which are both produced as a result of metabolic activity, can regulate the outcome of autophagy through acetylation-associated, posttranscriptional modifications of

and the uptake of both glucose and glutamine (Marino and Kroemer 2010). Activation of RAS is also able to induce autophagy in tumor cells (Guo et al. 2011; Lock et al. 2011; Yang and Kimmelman 2011). Tumor cells preferentially use aerobic glycolysis as an energy source, but cancer cells also depend on functional mitochondria for their growth and development. Autophagy might be essential to provide substrates for anaplerotic reactions, such as amino acids through protein degradation or lipids through the degradation of membrane organelles or of lipid droplets, in order to sustain mitochondrial metabolism (White 2012). As most of the glucose is consumed by glycolysis, glutamine becomes the main substrate for the mitochondrial TCA cycle and the generation of fatty acids and NADPH. Autophagy supports the profound metabolic rearrangements that cancer cells undergo, and this makes them highly dependent on autophagy for survival.

3.2.1 Autophagy and the Tumor Microenvironment

Tumor development (in particular that of solid tumors) depends on the exchanges that occur between cancer cells and their cellular and extracellular microenvironments [for reviews, see Mantovani et al. (2008), McAllister and Weinberg (2010)]. Various cell populations, including macrophages, lymphocytes, vascular cells, and carcinoma-associated fibroblasts, supplying growth factors, inflammatory cytokines, angiogenic factors, and elements of the extracellular matrix compose the tumoral stroma. The tumor cell microenvironment plays a major role in cancer progression by promoting neoangiogenesis, tissue remodeling, and the secretion of several factors (e.g., chemokines, cytokines, etc.) by immune cells. The role of the microenvironment in the regulation of autophagy in tumor cells in conjunction with the action of tumor cells on autophagy levels in cells in the surrounding stroma is of particular interest. The physiologically extreme conditions of the tumoral microenvironment (nutrient limitation/starvation, acidic pH, hypoxia, oxidative stress, immune responses) promote the autophagic response of cancer cells (i.e., survival and meeting the high energy demands of cancer cell metabolism). Tumor cells can also influence the autophagic activity of stromal cells. Understanding how autophagy regulates cancerous epithelial cells, fibroblasts, and immune cells and consequently the interactions between tumors and the stromal metabolism can be expected to provide new insights into the role of autophagy in the development and progression of tumors.

Several studies have shown that tumor cells release autophagy inducers into the microenvironment. These releases influence the autophagic activity of surrounding stromal cells, resulting in the secretion of high-energy metabolites (such as lactate

Fig. 3.2 (continued) autophagic key components that constitute the link between metabolic status and autophagy. *HATs* histone acetylases, *HDACs* histone deacetylases, *OAA* oxaloacetate, *PDK1* pyruvate dehydrogenase kinase 1, and the tricarboxylic acid (TCA) cycle

and ketones) as well as chemical building blocks such as amino acids (glutamine and nucleotides) that promote cancer development and progression (Marino and Kroemer 2010; Cheong et al. 2011). These catabolites stimulate the oxidative phosphorylation metabolism and mitochondrial biogenesis in epithelial cancer cells. A novel paradigm, known as the "autophagic tumor stroma model of cancer metabolism" or the "reverse Warburg effect," has recently been proposed to explain tumor metabolism (which is reprogrammed by oncogenic stress as mentioned above), in which the tumor stroma generates the fuel required for cancer growth (Pavlides et al. 2010). In this model, the induction of autophagy and the autophagic destruction of mitochondria force stroma cells to undergo glycolysis result in the production and transfer of energy-rich nutrients to anabolic tumor cells, which use them to fuel their mitochondrial metabolism (Eng et al. 2010; Martinez-Outschoorn et al. 2012). One autophagy inducer, ammonia, generated by amino acid catabolism including glutaminolysis, has been identified as a diffusible factor (Cheong et al. 2011); see Fig. 3.2. Ammonia stimulates autophagy in the neighboring stroma cells, leading to protein degradation, the generation of high glutamine levels, and the secretion of glutamine into the tumor microenvironment. Cancer cells convert glutamine into glutamate, thus releasing ammonia. Glutamate is further catabolized to α-ketoglutarate, a substrate of the tricarboxylic acid (TCA) cycle, which increases the mitochondrial activity of epithelial cancer cells. Epithelial cancer cells are less sensitive to ammonia, because TIGAR is upregulated (Ko et al. 2011). Several groups of researchers have demonstrated that autophagic cancer-associated fibroblasts (CAFs) produce a key source of energy-rich glutamine to "fuel" the mitochondrial activity of cancer cells. A vicious catabolic cycle is set up between the tumor stroma and anabolic tumor cell expansion; this highlights the metabolic coupling between epithelial and stroma cancer cells (in cancers of different histological types) (Kalluri and Zeisberg 2006). These studies show that glutamine differentially affects individual cell types within the tumor microenvironment. In tumor epithelial cells, glutamine increases mitochondrial biogenesis, providing protection against apoptosis and reducing autophagy. In contrast, glutamine decreases Caveolin-1 (Cav-1) expression in the stromal compartment of the tumor and increases autophagy. The loss of Cav-1 expression in cancer-associated fibroblasts is a marker of a lethal tumor-promoting microenvironment and is associated with poor prognosis in several types of cancers, such as advanced prostate cancer (Di Vizio et al. 2009), breast cancer (Sotgia et al. 2011; Pavlides et al. 2012), and metastatic melanoma (Wu et al. 2011). The use of autophagy inhibitors could not only promote tumor cell death by targeting tumor cells directly but also uncouple the epithelial and stromal compartments, leading to a decrease in epithelial mitochondrial activity.

3.2.2 Autophagy and Hypoxia

Limited access to oxygen owing to inadequate tissue perfusion, i.e., hypoxia, is a common feature of solid tumors. Hypoxia is a stimulus for inducing autophagy in order to promote tumor cell adaptation to anaerobic conditions (Martinez-Outschoorn et al. 2009; Mathew et al. 2007). The detection of markers of increased autophagic activity in hypoxic tumor tissues has been described (Rouschop et al. 2010). The transcriptional regulators that allow cells to adapt to hypoxic environments are the hypoxia-inducible factors (HIFs) HIF-1α and HIF-2α. They form a heterodimer with the constitutively expressed HIF-1β subunit. The oxygen-sensitive transcription factors HIF-1α and HIF-2α are tightly controlled through oxygen-dependent hydroxylation by prolyl hydroxylases. This hydroxylation leads to their degradation by the von Hippel-Lindau (VHL) ubiquitin-ligase under normoxic conditions. Under hypoxic conditions, the activity of prolyl hydroxylation is reduced, and both HIF-1α and HIF-2α are stabilized. These HIF complexes determine hypoxia-induced gene expression, including the production of the Bcl-2 homology-domain-3, which contains the proteins BNIP3 and BNIP3L (Semenza 2010). These BH3-only proapoptotic genes were initially described as promoters of cell death (Webster et al. 2005; Lee and Paik 2006), and now the BNIP3/BNIP3L proteins are known to destabilize inhibitory interactions between their antiapoptotic counterparts Bcl-xL/Bcl-2 and Beclin 1, leading to autophagy and promoting survival (Bellot et al. 2009). Autophagy induced by BNIP3 (known as mitophagy) results in the clearance of damaged mitochondria, which are a major source of cell-damaging reactive oxygen species (ROS), thus reducing ROS production (Xing et al. 2008). HIFs also regulate autophagy via TSC1/TSC2 activation and indirectly through a negative feedback mechanism, on MTORC1 activity (Rabinowitz and White 2010). Interestingly, activation of HIF by RAS impairs acetyl-CoA production by activating pyruvate dehydrogenase kinase 1 (PDK1), which inhibits pyruvate dehydrogenase (PDH). It is worth noting that RAS also impairs acetyl-CoA production through other mechanisms, including lactate dehydrogenase (LDH) stimulation, which depletes pyruvate, and by inhibiting liver kinase B1 (LKB1) and blocking AMP-activated protein kinase (AMPK) activation and preventing the mobilization of lipid stores and β-oxidation. Thus, RAS potentially leaves cells dependent on autophagy to provide substrates, such as amino acids and fatty acids, for acetyl-CoA biosynthesis (Fig. 3.2). Stroma cells, which also inhabit the same environment (oxidative stress, hypoxia) as tumor cells, contribute to the survival and proliferation of these cells (McAllister and Weinberg 2010). Indeed, ROS which are produced during hypoxia also induce the stabilization of HIF1/2α and the activation of NF-κB (a master regulator of inflammation) in CAFs (Eng and Abraham 2011). This stabilization leads to the autophagic degradation of Cav-1, a shift from mitochondrial oxidative phosphorylation towards aerobic glycolysis, with a loss of mitochondrial activity, and the increased production and release of L-lactate and ketone (Chiavarina et al. 2010). Furthermore, the ammonia produced by tumor cells could diffuse into the oxygen-depleted regions and thus help to sustain the survival of tumor cells (Eng et al. 2010).

3.3 Metabolism and Posttranslational Modification Regulation of Autophagy

Autophagy involves the hierarchical assembly and coordinated actions of products of the *Atg* family of genes. At least 30 members of the Atg (autophagy-related) protein family and their binding partners that orchestrate this degradative process have been identified. Autophagy is strictly controlled by posttranslational modifications, such as tyrosine or serine/threonine phosphorylation, lysine/arginine methylation, SUMOylation, lipidation, and ubiquitination of keys components of autophagy [for review, see McEwan and Dikic (2011)]. Henceforth, evidence is accumulating that protein lysine acetylation, which results from the transfer of an acetyl-group from acetyl-CoA to the ε-amino group of the lysine residue, is an evolutionarily conserved metabolic regulatory mechanism involved in coordinating various different metabolic pathways and autophagy (Zhao et al. 2010a). In addition, these posttranslational modifications concern key proteins, other than the histone proteins classically located both in the cytoplasm (including the core autophagy proteins, cytoskeletal proteins) and in the nucleus (such as transcriptional factors, histones), and modulate, thus influencing the rate at which autophagy occurs, which is known as the autophagic flux. This reaction is catalyzed by histone acetylases (HATs), which are also known as lysine acetyltransferases (such as p300), and the reverse reaction is accomplished by histone deacetylases (HDACs) or lysine deacetylases, which are zinc-dependent or NAD^+-dependent enzymes (such as SIRT1/2 sirtuins). The use of HDAC inhibitors and the studies of HATs and HDACs gain- and loss-of-function mutants highlight the pivotal role played by HATs and HDACs in autophagy regulation, where they act at multiple levels.

3.3.1 Posttranscriptional Modification Regulation at the Cytoplasmic Level

Studies have shown that resveratrol, an activator of the deacetylase Sirt1, and spermidine, an inhibitor of histone acetylases, influence the acetylation-modified proteome, induce autophagy, and increase longevity in yeast, nematodes, and flies (Eisenberg et al. 2009; Morselli et al. 2011). Changes in the acetylation status of >100 proteins that form part of the central network of autophagic regulators or executors have been identified after treatment with resveratrol and spermidine (Morselli et al. 2011). Since then, reversible cytoplasmic acetylation of core autophagy components, such as Atg5, Atg7, Atg8, and Atg12, has been reported to regulate autophagosome formation both in yeast and mammalian cells (McEwan and Dikic 2011), and it has been shown that acetylation of Atg proteins can either promote or inhibit their function in autophagy. In mammalian cells under nutrient-rich conditions, the acetyltransferase p300 directly interacts with Atg7 and acetylates the autophagy proteins Atg5, Atg7, Atg8, and Atg12 to inhibit autophagy

(Lee and Finkel 2009). In contrast, during starvation, p300 dissociates from Atg7, and the NAD^+-dependent deacetylase Sirt1 removes acetyl groups from Atg7, Atg5, Atg12, and Atg8, which allows autophagy to proceed (Lee et al. 2008). Some aspects of the phenotype of Sirt1 knockout mice resemble those of Atg5-knockout mice, which suggest that Sirt1-dependent deacetylation could be important for basal autophagy and neonatal survival (Lee et al. 2008). Another histone acetylase, Esa1p in *Saccharomyces cerevisiae* (Yi et al. 2012) and its mammalian ortholog TIP60 (Lin et al. 2012) have recently been shown to act as evolutionarily conserved regulators of autophagy by enhancing another posttranslational modification of Atg3 or ULK1, respectively, and protein lipidation of Atg8/LC3 (in the yeast). Acetylation of Lys183 enhanced the lipid-conjugating activity of Atg3p, and acetylation of Lys19 and Lys48 promoted the interaction between Atg3p and Atg8p, which is necessary for the conjugation of Atg8p to PE in yeast (Yamaguchi et al. 2010). The reverse reaction is accomplished by the deacetylase Rpd3, which contributes to attenuating the formation of autophagosomes during starvation. More interestingly, it has been demonstrated that Atg3 acetylation is subject to both spatial and temporal regulation during nitrogen starvation in the yeast. Thus, the acetyltransferase Esa1p/TIP60 has been demonstrated to be a positive regulator of autophagy in response to nitrogen deprivation in yeast and growth factor or serum deprivation in mammalian cells, respectively (Lin et al. 2012; Yi et al. 2012). Consistent with an essential role of TIP60 in autophagic induction, *TIP60*−/− mouse blastocysts failed to undergo implantation and died around embryonic day 3.5 at a time when autophagic activity is high during normal implantation (Hu et al. 2009; Mizushima and Komatsu 2011).

In addition, the acetylation of autophagy substrates can promote their lysosomal degradation [such as that of the cytotoxic huntingtin (HTT) protein] (Jeong et al. 2009), and the acetylation of microtubules and of the actin cytoskeleton, which provide support for intracellular transport/movement and also influence the occurrence/outcome of autophagy (Kochl et al. 2006). Microtubule stability and function are regulated by the reversible acetylation of α-tubulin mediated by HDAC682 and ELP3/KAT9 acetylases (Creppe et al. 2009), which also regulate the dynamics of actin (Zhang et al. 2007) and of SIRT2 deacetylase (North et al. 2003). In response to nutrient deprivation, tubulin acetylation on Lys40 increases in both the labile and stable microtubule fractions and promotes autophagy by favoring the activation and the association of key components for initiating autophagosome formation and maturation. Indeed, whereas the markers of phagophore/autophagosome formation (BECN1, class III PtdIns3K, WIPI1, ATG12–ATG5 and LC3-II) are specifically recruited on labile microtubules, mature autophagosomes (marked with LC3-II) can move along stable microtubules (Geeraert et al. 2010). In addition, tubulin acetylation is also essential for the fusion of autophagosomes to lysosomes (Kochl et al. 2006; Xie et al. 2010).

It is worth noting that HATs and HDACs are also subjected to reversible acetylation posttranscriptional modifications that provide a mechanism of fine-tuning and control of the activity of HATs and HDACs. For example, SIRT2

controls the self-acetylation of p300, which may also acetylate SIRT2 and inhibit its enzymatic activity (Black et al. 2008; Han et al. 2008).

3.3.2 *Posttranscriptional Modification Regulation at the Nuclear Level*

In addition to being regulated by cytoplasmic acetylation reactions, autophagy can also be regulated by the acetylation of nuclear proteins, which can influence the expression of genes encoding proteins involved in autophagy. These nuclear targets of acetylation-mediated regulation include transcription factors, such as Foxo3 (Kume et al. 2010), and histones (Eisenberg et al. 2009; Morselli et al. 2011). An example of the expression regulation of autophagy genes by histone acetylation is the increased expression of *ATG7* gene, resulting in spermidine-mediated histone hyperacetylation of the promoter region of the gene (Eisenberg et al. 2009). More recently, it has been demonstrated that the induction of autophagy is coupled to the reduction of histone H4 lysine 16 acetylation (H4K16ac) via the downregulation of the histone acetyltransferase hMOF (also designated KAT8 or MYST1), which regulates the outcome of autophagy and initiates a regulatory feedback loop (Fullgrabe et al. 2013). At a genome-wide level, H4K16 deacetylation is associated predominantly with the downregulation of autophagy-related genes (including genes belonging to the autophagic core machinery, such as ATG9A, GABARAPL2, MAP1LC3B, ULK1, ULK3, VMP1). Antagonizing the down-regulation of H4K16ac when autophagy is induced results in the promotion of cell death (associated with an overstimulation of autophagic flux), indicating that H4K16ac is a key determinant of survival versus death responses to the induction of autophagy.

In the case of the transcription factors belonging to the FOXO family members in mammalian cells, FOXO1 and FOXO3 have been shown to play important roles in regulating autophagy in skeletal and cardiac muscles by activating genes that are involved in autophagosome formation (such as *MAP1LC3*, *PIK3C3*, *GABARAPL1*, *ATG12*, *ATG4*, *BECN1*, *ULK1,* and *BNIP3)* (Mammucari et al. 2007; Sengupta et al. 2009; Zhao et al. 2010b). The multiple, posttranslational modifications (including acetylation) undergone by the FOXO transcription factors control their subcellular localization, DNA binding, and transcriptional properties (Van Der Heide et al. 2004; Boccitto and Kalb 2011). The acetylation of FOXO1 and FOXO3 is mediated by p300 acetyltransferase; it impairs their transcriptional activities and inhibits autophagy (Matsuzaki et al. 2005; Hariharan et al. 2010). The acetylation of FOXO1/3 promotes their subsequent phosphorylation by AKT1, leading to their dissociation from DNA, and subsequent nucleocytoplasmic transport (Matsuzaki et al. 2005; Tzivion et al. 2011). Under low-energy conditions, deacetylation of FOXO1/3 is mediated by the sirtuin deacetylases, such as SIRT1 and SIRT2, inducing the expression of genes that are involved in autophagosome

formation and also the gene that encodes PNPLA2, which is a major lipase involved in mobilizing fat from lipid droplets in mammals (Zimmermann et al. 2004; Gronke et al. 2005; Wang and Tong 2009). In addition, it has been shown that deacetylation-activated FOXO1 and FOXO3 activate the autophagic process in a transcription-independent manner by direct protein–protein interaction with ATG7 in response to serum deprivation in the context of human cancer cells (Zhao et al. 2010b).

3.3.3 Cross Talk Between Metabolism and Autophagy

Lysine protein acetylation can regulate the activity of the core components of autophagy, thus making it possible to couple the regulation of autophagy and the metabolic status of the cell. As already mentioned, autophagy ensures the maintenance of cellular energy/metabolic homeostasis by regulating intracellular storage, for example, by means of lipid mobilization (macrolipophagy) (Singh et al. 2009; Dong and Czaja 2011; Singh and Cuervo 2012). In a context of starvation, β-oxidation of fatty acids in the mitochondria results in lipid mobilization, leading to the production of NADH and the coenzyme of p300, i.e., acetyl-CoA. Increased levels of acetyl-CoA can produce a negative feedback by inhibiting starvation-induced autophagy/macrolipophagy via p300-mediated acetylation of autophagic components, and the associated lower level of NAD^+/NADH can lead to the inactivation of sirtuins, which in turn induces acetylation-associated autophagy, which constitutes the link between metabolic status and autophagy (see as below). In the context of Ras-driven cancers, which are more dependent on autophagy than normal cells in order to survive nutrient starvation or metabolic stress, Atg7 deficiency in lung tumor causes a shift from the development of carcinoma to oncocytomas which are rare and benign tumors that accumulate respiration-defective mitochondria (Guo et al. 2013). This effect has been attributed to defective mitochondrial fatty acid oxidation, which confirms that mitochondrial function maintained by Atg7 is critical for the metabolism and growth of Ras-driven NSCLC. In addition, it was also demonstrated in this study that autophagy suppresses the progression of K-Ras-induced lung tumors to oncocytomas, promoting the carcinoma fate.

Thus, acetylation joins phosphorylation, ubiquitination, and lipidation in the complex regulatory network controlling autophagy and constitutes the connection between autophagy and cellular metabolism. However, the mechanisms responsible for the recruitment of the HATs and HDACs during starvation or metabolic stress—thus controlling the acetylation of key components of autophagy and the initiation, duration, and magnitude of autophagy—remain topics for further investigation.

3.4 Autophagy, Metabolism, and Cancer Stem Cells

Recent work has highlighted the importance of the role played by autophagy in cancer stem-cell maintenance and tumor development. Cancer stem cells are a subpopulation of cells within tumors that are responsible for tumor recurrence and metastasis and tumor resistance to anticancer therapies. Autophagy seems to play a critical role in maintaining and regulating all the basic properties of both stem cells and cancer stem cells including survival, self-renewal, quiescence, differentiation, and proliferation (Cufi et al. 2011; Mortensen et al. 2011; Oliver et al. 2012; Salemi et al. 2012; Guan et al. 2013; Pan et al. 2013; Phadwal et al. 2013). We recently showed that autophagy is also crucial for the maintenance and tumorigenicity of cancer stem cells in breast cancer (Gong et al. 2012). Primary breast cancer stem cells have been shown to exhibit a higher rate of autophagy than their non-stem counterparts. Moreover, Beclin 1 is critical for the maintenance and tumor development of enriched cancer stem-cell tumors, in a xenograft mouse model, whereas its expression limits the development of classical xenografts (Gong et al. 2012). These findings indicate the existence of two separate, context-dependent autophagic programs that are regulated or respond in opposite ways by or to Beclin 1 (Koukourakis et al. 2010; Gong et al. 2012). Starvation- and hypoxia-related autophagy is a cytoprotective adaptive mechanism used by CSC to resist microenvironmental stresses (Guan et al. 2013). Cytogenetically abnormal, spheroid-forming, tumorigenic, and invasive neoplastic epithelial cells preexist in human breast ductal carcinoma in situ and require cellular autophagy to survive (Espina and Liotta 2011).

The suppression of autophagy by chloroquine abolishes spheroid outgrowth and survival in culture. These findings indicate that autophagy is necessary for the survival, growth, and invasion of the cytogenetically abnormal, tumorigenic DCIS cells (Espina and Liotta 2011; Guan et al. 2013). Similar data were obtained when knockdown of an essential gene of autophagy or a pharmacological inhibitor of autophagy, such as salinomycin, was employed (Yue et al. 2013).

Another study suggests that autophagy could promote the survival of pancreatic cancer stem cells (Singh et al. 2012). Moreover, autophagy also plays an essential role in glioblastoma stem-cell migration and invasion by modulating ATP metabolism and remodulating the subcellular structure, for instance, by mitochondrial fusion (Galavotti et al. 2013).

What is the possible contribution of autophagy to the metabolic shift of cancer cells towards enhanced glycolysis (the "Warburg effect") during the acquisition of stemness by CSC-like cell populations? This question calls for further investigation. If a "Warburg effect" does indeed play a causal role in the gain of stemlike properties by protecting tumor-initiating cells from the pro-senescent effects of mitochondrial respiration-induced oxidative stress, the ability of autophagy to functionally engage the glycolytic metabolite may generate a cellular state that is metabolically endowed with immortalization.

Paradoxically, autophagy appears to play two opposing roles: it acts as a facilitator of the "Warburg effect," but it also acts as an antagonist of the "Warburg effect." Both the inhibition and promotion of autophagy appear to impair the occurrence of cancer cells with tumor-initiating capacities to a similar extent. In normal tissues, autophagy-mediated damage mitigation may efficiently suppress tumorigenesis; conversely, macromolecular recycling may support CSC survival by buffering bioenergetic demands under stressful metabolic and microenvironmental conditions. Therefore, the activation of autophagy in normal tissues operates as a *bona fide* tumor-suppressive mechanism, whereas the inhibition of autophagy may be extremely beneficial for anti-CSC therapy in established tumors. However, both autophagy promoters (e.g., MTOR inhibitors) and autophagy inhibitors (e.g., chloroquine) block tumorigenesis and cancer progression by eliminating CSCs. The explanation of this "autophagy paradox" may lie in the interaction between tumor cells and adjacent, autophagic stromal cells, as described in Sect. 3.2 (Fig. 3.2).

To conclude, autophagy appears to play two opposing roles in tumorigenesis. The current hypothetical model describes autophagy as suppressing tumor initiation, but promoting tumor development and progression (Mathew et al. 2007; Koukourakis et al. 2010).

Conclusion

As our understanding of the biological functions of autophagy increases, the involvement of autophagy in cancer becomes a critical point of concern. The molecular cross talk between autophagy and cell death was initially considered to be a major determinant in the balanced role of autophagy in tumor suppression and tumor progression (Scarlatti et al. 2009). However, this relationship probably represents only aspect of the role of autophagy in cancer. The roles played by autophagy in tumor immunogenicity, inflammatory response, metabolism, proliferation, and the behavior of tumor-initiating cells are key questions for present and future studies (Michaud et al. 2011; Cheong et al. 2012; White 2012; Leone and Amaravadi 2013; Maes et al. 2013; Pan et al. 2013). It is now clear that the cross talk between autophagy and metabolism is an important aspect of cancer that contributes to the metabolic demand of cancer cells and also to the posttranslational modifications of proteins, i.e., acetylation, to modulate autophagic activity (McEwan and Dikic 2011). As discussed in this chapter, it is clear that autophagy plays an important role in the self-renewal of cancer stem cells. Whether autophagy also supports metabolism in cancer stem cells calls for further investigation (Shyh-Chang et al. 2013). Another important aspect is how basic knowledge about the autophagic process can be translated into therapeutic interventions. It is reasonable to speculate that autophagy modulation should be viewed as a potential therapeutic approach in cancer. Several phase I/II clinical trials are in progress using lysosome-inhibitor drugs, such as chloroquine, rapamycin, or hydroxychloroquine, alone or in combination

(continued)

with chemotherapy, to treat a range of hematological and solid tumors (Rubinsztein et al. 2012; Choi et al. 2013). However, it is not possible to exclude the possibility that the beneficial effects of these drugs could be independent of their blocking effect on the autophagic pathway. The development of more specific autophagy modulators, both for therapeutic investigations and to allow acute modulation of this process for cell biology and physiological studies, is a major challenge for the future.

Acknowledgments Work in P. Codogno's laboratory is supported by institutional funding from the Institut National de la Santé et de la Recherche Medicale (INSERM) and grants from the Agence Nationale de la Recherche (ANR) and the Institut National du Cancer (INCa).

References

Bellot G, Garcia-Medina R et al (2009) Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. Mol Cell Biol 29(10): 2570–2581

Bensaad K, Tsuruta A et al (2006) TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell 126(1):107–120

Black JC, Mosley A et al (2008) The SIRT2 deacetylase regulates autoacetylation of p300. Mol Cell 32(3):449–455

Boccitto M, Kalb RG (2011) Regulation of Foxo-dependent transcription by post-translational modifications. Curr Drug Targets 12(9):1303–1310

Botti J, Djavaheri-Mergny M et al (2006) Autophagy signaling and the cogwheels of cancer. Autophagy 2(2):67–73

Boya P, Reggiori F et al (2013) Emerging regulation and functions of autophagy. Nat Cell Biol 15(7):713–720

Cheong H, Lindsten T et al (2011) Ammonia-induced autophagy is independent of ULK1/ULK2 kinases. Proc Natl Acad Sci USA 108(27):11121–11126

Cheong H, Lu C et al (2012) Therapeutic targets in cancer cell metabolism and autophagy. Nat Biotechnol 30(7):671–678

Chiavarina B, Whitaker-Menezes D et al (2010) HIF1-alpha functions as a tumor promoter in cancer associated fibroblasts, and as a tumor suppressor in breast cancer cells: autophagy drives compartment-specific oncogenesis. Cell Cycle 9(17):3534–3551

Choi AM, Ryter SW et al (2013) Autophagy in human health and disease. N Engl J Med 368(7): 651–662

Creppe C, Malinouskaya L et al (2009) Elongator controls the migration and differentiation of cortical neurons through acetylation of alpha-tubulin. Cell 136(3):551–564

Cufi S, Vazquez-Martin A et al (2011) Autophagy positively regulates the CD44(+) CD24(-/low) breast cancer stem-like phenotype. Cell Cycle 10(22):3871–3885

Dang CV, Semenza GL (1999) Oncogenic alterations of metabolism. Trends Biochem Sci 24(2): 68–72

DeBerardinis RJ (2008) Is cancer a disease of abnormal cellular metabolism? New angles on an old idea. Genet Med 10(11):767–777

Deretic V, Levine B (2009) Autophagy, immunity, and microbial adaptations. Cell Host Microbe 5(6):527–549

Di Vizio D, Morello M et al (2009) An absence of stromal caveolin-1 is associated with advanced prostate cancer, metastatic disease and epithelial Akt activation. Cell Cycle 8(15):2420–2424
Dong H, Czaja MJ (2011) Regulation of lipid droplets by autophagy. Trends Endocrinol Metab 22(6):234–240
Eisenberg T, Knauer H et al (2009) Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 11(11):1305–1314
Eng CH, Abraham RT (2011) The autophagy conundrum in cancer: influence of tumorigenic metabolic reprogramming. Oncogene 30(47):4687–4696
Eng CH, Yu K et al (2010) Ammonia derived from glutaminolysis is a diffusible regulator of autophagy. Sci Signal 3(119):ra31
Espina V, Liotta LA (2011) What is the malignant nature of human ductal carcinoma in situ? Nat Rev Cancer 11(1):68–75
Fullgrabe J, Lynch-Day MA et al (2013) The histone H4 lysine 16 acetyltransferase hMOF regulates the outcome of autophagy. Nature 500(7463):468–471
Galavotti S, Bartesaghi S et al (2013) The autophagy-associated factors DRAM1 and p62 regulate cell migration and invasion in glioblastoma stem cells. Oncogene 32(6):699–712
Geeraert C, Ratier A et al (2010) Starvation-induced hyperacetylation of tubulin is required for the stimulation of autophagy by nutrient deprivation. J Biol Chem 285(31):24184–24194
Gong C, Bauvy C et al (2013) Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. Oncogene 32(18):2261–2272, 2272e 1–11
Gong C, Song E et al (2012) The roles of BECN1 and autophagy in cancer are context dependent. Autophagy 8(12):1853–1855
Gordan JD, Thompson CB et al (2007) HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. Cancer Cell 12(2):108–113
Gronke S, Mildner A et al (2005) Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. Cell Metab 1(5):323–330
Guan JL, Simon AK et al (2013) Autophagy in stem cells. Autophagy 9(6):830–849
Guo JY, Chen HY et al (2011) Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev 25(5):460–470
Guo JY, Karsli-Uzunbas G et al (2013) Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. Genes Dev 27(13):1447–1461
Hamasaki M, Shibutani ST et al (2013) Up-to-date membrane biogenesis in the autophagosome formation. Curr Opin Cell Biol 25:455–460
Han Y, Jin YH et al (2008) Acetylation of Sirt2 by p300 attenuates its deacetylase activity. Biochem Biophys Res Commun 375(4):576–580
Hariharan N, Maejima Y et al (2010) Deacetylation of FoxO by Sirt1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. Circ Res 107(12):1470–1482
Hu Y, Fisher JB et al (2009) Homozygous disruption of the Tip60 gene causes early embryonic lethality. Dev Dyn 238(11):2912–2921
Jeong H, Then F et al (2009) Acetylation targets mutant huntingtin to autophagosomes for degradation. Cell 137(1):60–72
Kalluri R, Zeisberg M (2006) Fibroblasts in cancer. Nat Rev Cancer 6(5):392–401
Kawauchi K, Araki K et al (2008) p53 regulates glucose metabolism through an IKK-NF-kappaB pathway and inhibits cell transformation. Nat Cell Biol 10(5):611–618
Ko YH, Lin Z et al (2011) Glutamine fuels a vicious cycle of autophagy in the tumor stroma and oxidative mitochondrial metabolism in epithelial cancer cells: implications for preventing chemotherapy resistance. Cancer Biol Ther 12(12):1085–1097
Kochl R, Hu XW et al (2006) Microtubules facilitate autophagosome formation and fusion of autophagosomes with endosomes. Traffic 7(2):129–145
Koukourakis MI, Giatromanolaki A et al (2010) Beclin 1 over- and underexpression in colorectal cancer: distinct patterns relate to prognosis and tumour hypoxia. Br J Cancer 103(8): 1209–1214

Kroemer G, Marino G et al (2010) Autophagy and the integrated stress response. Mol Cell 40 (2):280–293
Kume S, Uzu T et al (2010) Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. J Clin Invest 120(4):1043–1055
Lee H, Paik SG (2006) Regulation of BNIP3 in normal and cancer cells. Mol Cells 21(1):1–6
Lee IH, Cao L et al (2008) A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. Proc Natl Acad Sci USA 105(9):3374–3379
Lee IH, Finkel T (2009) Regulation of autophagy by the p300 acetyltransferase. J Biol Chem 284(10):6322–6328
Leone RD, Amaravadi RK (2013) Autophagy: a targetable linchpin of cancer cell metabolism. Trends Endocrinol Metab 24(4):209–217
Lin SY, Li TY et al (2012) GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy. Science 336(6080):477–481
Liu EY, Ryan KM (2012) Autophagy and cancer-issues we need to digest. J Cell Sci 125(Pt 10): 2349–2358
Lock R, Roy S et al (2011) Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. Mol Biol Cell 22(2):165–178
Lorin S, Hamai A et al (2013) Autophagy regulation and its role in cancer. Semin Cancer Biol 23: 361–379
Maes H, Rubio N et al (2013) Autophagy: shaping the tumor microenvironment and therapeutic response. Trends Mol Med 19(7):428–446
Maiuri MC, Tasdemir E et al (2009) Control of autophagy by oncogenes and tumor suppressor genes. Cell Death Differ 16(1):87–93
Mammucari C, Milan G et al (2007) FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metab 6(6):458–471
Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. Cell 129(7): 1261–1274
Mantovani A, Romero P et al (2008) Tumour immunity: effector response to tumour and role of the microenvironment. Lancet 371(9614):771–783
Marino G, Kroemer G (2010) Ammonia: a diffusible factor released by proliferating cells that induces autophagy. Sci Signal 3(124):pe19
Martinez-Outschoorn UE, Sotgia F et al (2012) Power surge: supporting cells "fuel" cancer cell mitochondria. Cell Metab 15(1):4–5
Martinez-Outschoorn UE, Trimmer C et al (2009) Autophagy in cancer associated fibroblasts promotes tumor cell survival: role of hypoxia, HIF1 induction and NFkappaB activation in the tumor stromal microenvironment. Cell Cycle 9(17):3515–3533
Mathew R, Karantza-Wadsworth V et al (2007) Role of autophagy in cancer. Nat Rev Cancer 7(12):961–967
Matoba S, Kang JG et al (2006) p53 regulates mitochondrial respiration. Science 312(5780): 1650–1653
Matsuzaki H, Daitoku H et al (2005) Acetylation of Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. Proc Natl Acad Sci USA 102(32):11278–11283
McAllister SS, Weinberg RA (2010) Tumor-host interactions: a far-reaching relationship. J Clin Oncol 28(26):4022–4028
McEwan DG, Dikic I (2011) The three musketeers of autophagy: phosphorylation, ubiquitylation and acetylation. Trends Cell Biol 21(4):195–201
Michaud M, Martins I et al (2011) Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. Science 334(6062):1573–1577
Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. Cell 147(4): 728–741
Mizushima N, Levine B et al (2008) Autophagy fights disease through cellular self-digestion. Nature 451(7182):1069–1075

Mizushima N, Yoshimori T et al (2011) The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol 27:107–132
Moreau K, Renna M et al (2013) Connections between SNAREs and autophagy. Trends Biochem Sci 38(2):57–63
Morselli E, Marino G et al (2011) Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. J Cell Biol 192(4):615–629
Mortensen M, Soilleux EJ et al (2011) The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. J Exp Med 208(3):455–467
North BJ, Marshall BL et al (2003) The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. Mol Cell 11(2):437–444
Oliver L, Hue E et al (2012) Basal autophagy decreased during the differentiation of human adult mesenchymal stem cells. Stem Cells Dev 21(15):2779–2788
Pan H, Cai N et al (2013) Autophagic control of cell 'stemness'. EMBO Mol Med 5(3):327–331
Pavlides S, Tsirigos A et al (2010) The autophagic tumor stroma model of cancer: role of oxidative stress and ketone production in fueling tumor cell metabolism. Cell Cycle 9(17):3485–3505
Pavlides S, Vera I et al (2012) Warburg meets autophagy: cancer-associated fibroblasts accelerate tumor growth and metastasis via oxidative stress, mitophagy, and aerobic glycolysis. Antioxid Redox Signal 16(11):1264–1284
Phadwal K, Watson AS et al (2013) Tightrope act: autophagy in stem cell renewal, differentiation, proliferation, and aging. Cell Mol Life Sci 70(1):89–103
Rabinowitz JD, White E (2010) Autophagy and metabolism. Science 330(6009):1344–1348
Ravikumar B, Sarkar S et al (2010) Regulation of mammalian autophagy in physiology and pathophysiology. Physiol Rev 90(4):1383–1435
Rouschop KM, van den Beucken T et al (2010) The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. J Clin Invest 120(1):127–141
Rubinsztein DC, Codogno P et al (2012) Autophagy modulation as a potential therapeutic target for diverse diseases. Nat Rev Drug Discov 11(9):709–730
Salemi S, Yousefi S et al (2012) Autophagy is required for self-renewal and differentiation of adult human stem cells. Cell Res 22(2):432–435
Scarlatti F, Granata R et al (2009) Does autophagy have a license to kill mammalian cells? Cell Death Differ 16(1):12–20
Semenza GL (2010) Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 29(5):625–634
Sengupta A, Molkentin JD et al (2009) FoxO transcription factors promote autophagy in cardiomyocytes. J Biol Chem 284(41):28319–28331
Shyh-Chang N, Daley GQ et al (2013) Stem cell metabolism in tissue development and aging. Development 140(12):2535–2547
Singh BN, Kumar D et al (2012) Rottlerin induces autophagy which leads to apoptotic cell death through inhibition of PI3K/Akt/mTOR pathway in human pancreatic cancer stem cells. Biochem Pharmacol 84(9):1154–1163
Singh R, Cuervo AM (2012) Lipophagy: connecting autophagy and lipid metabolism. Int J Cell Biol 2012:282041
Singh R, Kaushik S et al (2009) Autophagy regulates lipid metabolism. Nature 458(7242): 1131–1135
Sotgia F, Martinez-Outschoorn UE et al (2011) Understanding the Warburg effect and the prognostic value of stromal caveolin-1 as a marker of a lethal tumor microenvironment. Breast Cancer Res 13(4):213
Tzivion G, Dobson M et al (2011) FoxO transcription factors; regulation by AKT and 14-3-3 proteins. Biochim Biophys Acta 1813(11):1938–1945
Van Der Heide LP, Hoekman MF et al (2004) The ins and outs of FoxO shuttling: mechanisms of FoxO translocation and transcriptional regulation. Biochem J 380(Pt 2):297–309

Wang F, Tong Q (2009) SIRT2 suppresses adipocyte differentiation by deacetylating FOXO1 and enhancing FOXO1's repressive interaction with PPARgamma. Mol Biol Cell 20(3):801–808
Webster KA, Graham RM et al (2005) BNip3 and signal-specific programmed death in the heart. J Mol Cell Cardiol 38(1):35–45
White E (2012) Deconvoluting the context-dependent role for autophagy in cancer. Nat Rev Cancer 12(6):401–410
Wu KN, Queenan M et al (2011) Loss of stromal caveolin-1 expression in malignant melanoma metastases predicts poor survival. Cell Cycle 10(24):4250–4255
Xie R, Nguyen S et al (2010) Acetylated microtubules are required for fusion of autophagosomes with lysosomes. BMC Cell Biol 11:89
Xing C, Zhu B et al (2008) Class I phosphatidylinositol 3-kinase inhibitor LY294002 activates autophagy and induces apoptosis through p53 pathway in gastric cancer cell line SGC7901. Acta Biochim Biophys Sin (Shanghai) 40(3):194–201
Yamaguchi M, Noda NN et al (2010) Autophagy-related protein 8 (Atg8) family interacting motif in Atg3 mediates the Atg3-Atg8 interaction and is crucial for the cytoplasm-to-vacuole targeting pathway. J Biol Chem 285(38):29599–29607
Yang S, Kimmelman AC (2011) A critical role for autophagy in pancreatic cancer. Autophagy 7(8):912–913
Yang Z, Klionsky DJ (2010) Eaten alive: a history of macroautophagy. Nat Cell Biol 12(9): 814–822
Yi C, Ma M et al (2012) Function and molecular mechanism of acetylation in autophagy regulation. Science 336(6080):474–477
Yue W, Hamai A et al (2013) Inhibition of the autophagic flux by salinomycin in breast cancer stem-like/progenitor cells interferes with their maintenance. Autophagy 9(5):714–729
Zhang X, Yuan Z et al (2007) HDAC6 modulates cell motility by altering the acetylation level of cortactin. Mol Cell 27(2):197–213
Zhao S, Xu W et al (2010a) Regulation of cellular metabolism by protein lysine acetylation. Science 327(5968):1000–1004
Zhao Y, Yang J et al (2010b) Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity. Nat Cell Biol 12(7):665–675
Zimmermann R, Strauss JG et al (2004) Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 306(5700):1383–1386