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Autophagy and cancer cell metabolism

Fred Lozy^{a,b} and Vassiliki Karantza^{a,b,c,*}

^aUniversity of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, United States

^bThe Cancer Institute of New Jersey, 195 Little Albany Street, New Brunswick, NJ 08903, United States

^cDivision of Medical Oncology, Department of Internal Medicine, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, United States

Abstract

Autophagy is a catabolic process involving lysosomal turnover of proteins and organelles for maintenance of cellular homeostasis and mitigation of metabolic stress. Autophagy defects are linked to diseases, such as liver failure, neurodegeneration, inflammatory bowel disease, aging and cancer. The role of autophagy in tumorigenesis is complex and likely context-dependent. Human breast, ovarian and prostate cancers have allelic deletions of the essential autophagy regulator *BECN1* and *Becn1*^{+/-} and other autophagy-deficient transgenic mice are tumor-prone, whereas tumors with constitutive Ras activation, including human pancreatic cancers, upregulate basal autophagy and are commonly addicted to this pathway for survival and growth; furthermore, autophagy suppression by *Fip200* deletion compromises PyMT-induced mammary tumorigenesis. The double-edged sword function of autophagy in cancer has been attributed to both cell- and non-cell-autonomous mechanisms, as autophagy defects promote cancer progression in association with oxidative and ER stress, DNA damage accumulation, genomic instability and persistence of inflammation, while functional autophagy enables cancer cell survival under stress and likely contributes to treatment resistance. In this review, we will focus on the intimate link between autophagy and cancer cell metabolism, a topic of growing interest in recent years, which has been recognized as highly clinically relevant and has become the focus of intense investigation in translational cancer research. Many tumor-associated conditions, including intermittent oxygen and nutrient deprivation, oxidative stress, fast growth and cell death suppression, modulate, in parallel and in interconnected ways, both cellular metabolism and autophagy to enable cancer cells to rapidly adapt to environmental stressors, maintain uncontrolled proliferation and evade the toxic effects of radiation and/or chemotherapy. Elucidating the interplay between autophagy and tumor cell metabolism will provide unique opportunities to identify new therapeutic targets and develop synthetically lethal treatment strategies that preferentially target cancer cells, while sparing normal tissues.

Keywords

Autophagy; Cancer cell metabolism; Warburg effect; Oxidative phosphorylation; Glycolysis

1. Introduction

Autophagy is a highly conserved catabolic process with critical functions in maintenance of cellular homeostasis under normal growth conditions and in preservation of cell viability under stress [1,2]. Cellular self-eating involves entrapment of cytoplasm and organelles in double-membrane vesicles called autophagosomes, followed by “cargo” degradation in lysosomes, thereby recycling energy and building blocks for the synthesis of new biomolecules. Autophagy can be non-selective, leading to lysosomal processing of bulk cytoplasmic material, or selective, involving targeted degradation of specific proteins and/or organelles, such as mitochondria (mitophagy), ribosomes (ribophagy), or lipids (lipophagy) [2,3]. In recent years, the mechanisms regulating selective autophagy have become the focus of intense investigation. Defects in basal autophagy result in toxic accumulation of protein aggregates and damaged organelles, whereas defects in stress-induced autophagy primarily limit cell survival [4]. In either case, cellular fitness is compromised, potentially resulting in impairment of genome integrity and stability, and ultimately etiologically linking defective autophagy to disease states, such as cancer, aging, liver disease, and neurodegeneration [3,5–10].

The role of autophagy in tumorigenesis is complex and likely tissue- and genetic context-dependent. The connection between autophagy and cancer cell metabolism is a topic of great interest and high potential for clinical relevance in cancer research, as metabolically stressed tumor cells rely on autophagy for survival and the reprogramming of their metabolism to accommodate rapid cell growth and proliferation. To this purpose, specific catabolic reactions, such as aerobic glycolysis and glutaminolysis, are upregulated to provide energy and, even more importantly, the building elements for new protein, nucleic acid and lipid production (Fig. 1). This review will summarize our current knowledge on the relationship between cancer cell metabolism and autophagy, incorporating examples of how different oncogenes impact these processes and pointing out opportunities for therapeutic intervention and pharmacologic modulation of this interaction for clinical benefit.

2. Autophagy regulation

In eukaryotic cells, the mammalian target of rapamycin complex 1 (mTORC1) acts as the central sensor of nutrient availability and a major regulator of autophagy. mTORC1 is activated under nutrient-rich conditions and suppresses autophagy by phosphorylating two components of the Unc-51-like kinase (ULK1) complex, ULK1 and Atg13, resulting in their inactivation and the inhibition of autophagosome nucleation [11,12]. Reversely, growth factor depletion or pharmacologic mTORC1 inhibition suppresses the high energy-demanding process of mRNA translation and stimulates autophagy [13] by several mechanisms. Glucose deprivation leads to AMP accumulation and AMP kinase (AMPK) activation, which in turn upregulates autophagy by (1) phosphorylating the tuberous sclerosis complex (TSC) 2, which inhibits the GTPase Rheb and its downstream effector mTORC1 [14,15], and (2) directly activating the ULK1 complex itself [16]; hypoxia induces expression of hypoxia-inducible factors (HIFs), which activate TSC1, again resulting in Rheb and mTORC1 activity suppression [17]; amino acid depletion interferes with Rag GTPase-mediated mTORC1 translocation to the lysosomal surface and subsequent activation by Rheb [18–20]. Less-well-understood, mTOR-independent pathways of autophagy regulation also exist. In muscle, lack of amino acids, particularly leucine, induces autophagy via FoxO-dependent transcription of autophagy-related-genes, such as microtubule-associated protein 1 light chain 3b (LC3) and BCL2/adenovirus E1B protein-interacting protein (BNIP3) [21,22]; ammonia, a glutaminolysis byproduct, stimulates autophagy by a mechanism dependent on Atg5, but not mTOR or ULK1 [23,24]; finally,

numerous drugs have been reported to induce autophagy independently of mTOR inactivation [25,26].

3. The many faces and functions of autophagy

3.1. Cellular homeostasis and cell survival

Genetic manipulation of essential autophagy genes in mice has illustrated the importance of functional autophagy for cell survival under stress and for the maintenance of cellular homeostasis. For example, biallelic *Becn1* (*Atg6*) deletion results in embryonic lethality, whereas *Atg5*^{-/-} or *Atg7*^{-/-} pups are born, but succumb to neonatal starvation [7,8,27,28]. In the background of impaired apoptosis, autophagy defects compromise cell viability in response to growth factor deprivation or metabolic stress [29,30]. Quite importantly, this finding is of greater relevance to cancer – as compared to normal-cells, as tumors frequently exhibit high metabolic demands due to oncogene activation and repetitive outstripping of their blood supply. Indeed, autophagy induction is spatially localized to hypoxic tumor regions [5,29] and may signify differential cancer cell dependence on autophagy for survival under stressful conditions. Both radiation and chemotherapy commonly induce autophagy [31], primarily as a stress-relieving and prosurvival function that is critical for cancer cell viability; in such cases, genetic or pharmacologic autophagy inhibition preferentially sensitizes cancer cells to treatment [32,33]. These findings are currently under clinical investigation with several studies examining whether pharmacologic inhibition of autophagy with the anti-malaria and non-specific autophagy inhibitor hydroxychloroquine augments the efficacy of standard anticancer regimens [31–34].

The role of autophagy in cellular homeostasis has best been demonstrated by studies on brain and liver pathophysiology: mice with brain-specific *Atg5* or *Atg7* deletion exhibit neuronal degeneration with a buildup of damaged mitochondria and protein aggregates, which are hallmarks of Huntington's and Parkinson's diseases [35,36]; p62-containing protein aggregates are found in liver-specific *Atg7*-deficient mice, exhibiting extensive liver damage and accelerated hepatocyte cell death [37]. Furthermore, autophagy-deficient mice display impaired adipocyte differentiation and increased insulin sensitivity [38,39].

3.2. Tumorigenesis

Autophagy was first linked to tumorigenesis when the essential autophagy regulator *BECLIN1* (*BECN1*) was reported allelically deleted in many breast, ovarian and prostate cancers, suggesting that autophagy might be a tumor suppressor mechanism [40]. In support of this hypothesis, re-expression of the BECN1 protein restores autophagy and suppresses tumorigenesis by breast cancer cell lines [41], and monoallelic *Becn1* deletion results in liver and lung carcinomas, lymphomas, and mammary hyperplasias in aging mice [27,28]. Defects in other autophagy regulators have also been associated with a protumorigenic phenotype: *Atg4C*^{-/-} mice show increased susceptibility to fibrosarcomas induced by chemical carcinogens [42]; complete loss of UVRAG-binding protein BIF-1, a positive regulator of autophagy and a BECN1-interacting protein, in mice causes increased spontaneous tumor formation [43]; apoptosis-defective *Atg5*^{-/-} immortalized baby mouse kidney (iBMK) cells [44] and *Becn1*^{+/-} immortalized mouse mammary epithelial cells (iMMECs) [30] are more tumorigenic than their autophagy-competent counterparts in nude mice; systemic mosaic *Atg5* deletion and liver-specific *Atg7* deletion both result in benign liver adenomas originating from autophagy-deficient hepatocytes [45].

The conundrum on the role of autophagy in tumorigenesis arises from the superficially contradictory functions of autophagy as both a cancer cell survival and a tumor suppressive mechanism. However, such a paradox has a closely related precedent in the role of DNA repair proteins in cancer [46] and, similar to that, several explanations reconciling the

double-edged sword role of autophagy in cancer have been proposed. Cell-autonomous mechanisms include the enhanced DNA damage and the genomic instability exhibited by autophagy-deficient cells, secondary to increased ROS levels and oxidative stress in association with aberrant accumulation of organelles, such as damaged mitochondria [47]. Protein aggregates are also dependent on autophagic degradation for clearance, and when autophagy is defective, both protein aggregates and their autophagosome cargo receptor p62—itsself normally degraded by autophagy—accumulate and trigger a cascade of cellular events, including ROS production, ER stress, and DNA damage response activation. Ectopic p62 expression in autophagy-competent cells leads to protein aggregate formation, causes oxidative stress and promotes tumorigenesis, thus mimicking the autophagy-deficiency phenotype, while p62 knockdown eliminates protein aggregates and inhibits tumor formation [48]. Liver-specific autophagy defects result in p62-containing protein aggregates, which cause liver damage and inhibit the NF- κ B pathway resulting in a vicious cell death-neighboring cell proliferation cycle that promotes hepatocellular carcinoma (HCC) [48]. Similarly, defective autophagy leads to accumulation of alpha-1-antitrypsin (AT) aggregates in liver and lung, which in turn render cells resistant to apoptosis and cause inflammation and oxidative stress, all conditions potentially fueling cancer progression [49,50].

Inflammation has independently been reported as a non-cell-autonomous mechanism by which defective autophagy contributes to tumorigenesis, as metabolically stressed cells with combined apoptosis-and-autophagy defects undergo necrotic cell death resulting in an inflammatory microenvironment [29], itself a risk factor for cancer development, as exemplified by the increased cancer incidence associated with chronic pancreatitis and inflammatory bowel disease (IBD) [51,52]. In support of this hypothesis, *Atg5*^{-/-} or *Atg7*^{-/-} mice or mice hypomorphic for *Atg16l1* exhibit intestinal Paneth cell abnormalities resembling Crohn's disease; furthermore, autophagy defects have been documented in intestinal lesions from IBD patients [53,54].

Along similar lines, autophagy defects may promote tumorigenesis by compromising antigen cross presentation and, thus, adaptive immunity. Melanoma cells and human embryonic kidney cells show reduced antigen cross presentation and T-cell proliferation when autophagy is inhibited by 3-methyladenine or knockdown of BECN1 or ATG12, whereas the reverse trend is observed when autophagy is upregulated by rapamycin or starvation [55]. Furthermore, autophagy-deficient T-cells proliferate less than their autophagy-competent counterparts and are susceptible to apoptosis [56,57], whereas thymic epithelial cells with impaired autophagy do not properly choose major histocompatibility complex class II (MHC-II) molecules, commonly selecting host rather than foreign antigens and causing a strong and persistent autoimmune response and a tumor-enabling microenvironment [58].

In contrast to the unifying message of the studies summarized above presenting autophagy as a bona fide or potential tumor suppressive mechanism, more recent cancer mouse model studies have brought attention to the tumor-promoting effects of autophagy. In a K-Ras-driven genetically engineered mouse model of pancreatic ductal adenocarcinoma (PDAC), treatment of advanced pancreatic intraepithelial neoplasias or focal PDAC with the non-specific autophagy inhibitor chloroquine, delays tumor progression and increases mouse survival [59], whereas in the first report of a mouse model combining defective autophagy with a well-characterized oncogenic function, autophagy inhibition by *Fip200* ablation suppresses PyMT-driven mammary tumor initiation and progression in association with decreased cyclin D1 expression, induction of interferon (IFN)-responsive genes, effector T-cell tumor infiltration and increased production of cytokines, such as CXCL10 [60].

Clearly, autophagy plays important roles in the maintenance of cell survival and homeostasis, particularly for metabolically active, and thus very sensitive to disturbances of energetic balance, cancer cells. The role of autophagy in tumorigenesis is likely dependent on many parameters, including (1) tumor stage, such as initiation, progression, metastasis, and/or development of treatment resistance, (2) tissue involved, and (3) accompanying tumor-promoting genetic changes and order of their appearance. Elucidating the nuances of the context-specific role of autophagy in cancer will expand our current understanding of the pathophysiology of this devastating disease and will enable us to rationally design improved anticancer regimens involving pharmacologic manipulation of autophagy for maximal clinical benefit.

4. Autophagy and cancer cell metabolism

4.1. Autophagy is essential to support cancer cell growth and metabolism

Metabolic reprogramming in cancer or the “Warburg effect” refers to the rather unique characteristic of cancer cells to increase the speed, but with a decrease in efficiency, of energy production by upregulating aerobic glycolysis and reducing electron transport chain activity in mitochondria, respectively [61]. Several oncogenic driver mutations, such as those involving Ras or Akt, activate the Warburg effect by increasing glucose uptake, transcription of enzymes involved in glucose metabolism, and the rate of glycolysis [62,63]. Several oncogenes have also been reported to inhibit autophagy, potentially through mTOR activation, supporting the hypothesis that catabolic processes are often suppressed in tumors to facilitate cell mass accumulation [64]. However, strong evidence has emerged that, in at least one case, autophagy is required during the transformation process. In contrast to the majority of oncogenes examined so far, K-Ras not only induces basal autophagy, but also depends on functional autophagy for tumor growth, as reported by several recent studies: autophagy defects attenuate Ras-mediated adhesion-independent transformation and proliferation, decrease glycolysis, likely interfering with the increased glycolytic requirements imposed by Ras activation, and render Ras-expressing cancer cells insensitive to glucose limitation [65]; *Atg5*^{-/-} or *Atg7*^{-/-} K-Ras expressing iBMK cells show compromised oxidative phosphorylation by tricarboxylic acid (TCA) cycle intermediate depletion and damaged mitochondria accumulation, ultimately resulting in suppression of Ras-induced tumorigenesis [66]; similarly, stable knockdown of ATG5 or ATG7 in Ras-expressing human breast cancer cells inhibits cell growth in soft agar and tumor formation in nude mice [67]; genetic or pharmacologic inhibition of autophagy in human pancreatic cancer cell lines, almost ubiquitously harboring Ras mutations and showing elevated autophagy under basal conditions, results in decreased mitochondrial oxidative phosphorylation and tumor cell growth inhibition in vitro and in xenograft tumor studies and genetic mouse models in vivo [59]; finally, autophagy inhibition by *Fip200* deletion impairs PyMT-induced mammary tumorigenesis and is associated with decreased intratumoral glycolysis [60]. Thus, Ras-driven tumorigenesis appears “addicted to autophagy” for essential metabolic support and maintenance of rapid tumor growth, a clinically very important finding that opens the door to the investigation of autophagy inhibitors as treatment response modulators in pancreatic and other Ras-mutant human malignancies. Although the polyoma virus has not been etiologically linked to human breast cancer, the findings of the mammary *Fip200* ablation indicate that potentially other potent oncogenes rely on autophagy for tumor initiation and progression.

4.2. Metabolic reprogramming in cancer favors autophagy induction

Activation of oncogenes and loss of tumor suppressor genes in cancer, such as PTEN or p53, commonly upregulate glycolysis and other cell survival pathways for optimal cell growth and proliferation. For example, the p53 target TP53-induced glycolysis and apoptosis

regulator (TIGAR) normally inhibits glycolytic activity and channels glucose metabolism to the pentose phosphate pathway (PPP) for increased production of NADPH, in turn required for fatty acid synthesis, regeneration of reduced glutathione, ROS reduction and autophagy suppression. Thus, p53 deletion results in activation of both glycolysis and autophagy [68].

Although mitochondrial dysfunction was initially considered a tumor cell characteristic and the major reason behind the Warburg effect, more recent studies have demonstrated that cancer growth depends on functional mitochondria. However, since glucose is mostly used for aerobic glycolysis, glutamine becomes a major substrate for the mitochondrial TCA cycle and the generation of NADPH and fatty acids. Tumor cells, such as those with activated Myc, upregulate glutamine transporters and enzymes, and are dependent on glutaminolysis for survival [69]. Ammonia produced by glutaminolysis in turn increased basal autophagy, protects cancer cells from by TNF α -induced apoptosis, and limits proliferation under metabolic stress [23]. Glutamine-deficient conditions result in decreased autophagic activity, while ammonia rescues cell death from long-term glutamine withdrawal through induction of autophagy [70]. Interestingly, ammonia has been discovered in tumor interstitial fluids, perhaps as a diffusible signal to hypoxic tumor regions to increase autophagy and preserve cancer cell viability (Fig. 1) [24].

Autophagy-mediated survival under hypoxic conditions is indispensable for cancer growth and progression. Hypoxia induces ROS production and, at the same time, autophagy for the elimination of damaged mitochondria, which could produce even more ROS with detrimental effects for the cell [71,72]. HIF-1 α is stabilized under hypoxic conditions and induces transcription of several genes enabling cell survival, such as the positive autophagy regulator BNIP3, which induces mitophagy to clear impaired mitochondria [17]. HIF-1 also has the ability to suppress the mTOR pathway through its activation of REDD1, which represses mTORC1, also stimulating autophagy [73]. Furthermore, hypoxia stimulates autophagy in a ROS- and HIF-1-independent mechanism involving PERK activation in response to endoplasmic reticulum stress and resulting in the transcriptional upregulation of the autophagy genes *Atg5* and *LC3* [74].

4.3. Tumor microenvironment, autophagy, and metabolism

In the recent years, many studies have focused on the tumor microenvironment, i.e. the relationship between cancer and its surrounding stroma, which plays an important role in every step of tumorigenesis, from initiation to metastasis, and may even contribute to treatment resistance. In a crosstalk between cancer and stromal cells, growth factors and cytokines are exchanged, ultimately facilitating tumor growth and progression [75]. Such a critical interaction taking place in the tumor microenvironment is the epithelial-stromal metabolic coupling or “reverse Warburg effect”, whereby cancer cells “hijack” neighboring stromal cells or tumor-associated fibroblasts (TAFs) and use nutrients produced by TAFs for sustaining tumor cell metabolism (Fig. 2). More specifically, oncogene activation- and high metabolism-associated ROS production by cancer cells causes oxidative stress in TAFs, which undergo autophagy and mitophagy, loss of mitochondrial function, and increase in aerobic glycolysis, releasing nutrients such as lactate and ketones, which in turn “fuel” mitochondrial biogenesis and oxidative phosphorylation in cancer cells in a self-perpetuating vicious cycle. It is also postulated that TAF-generated ROS and the resultant oxidative stress cause DNA damage, genomic instability, and aneuploidy in neighboring cancer cells, thus promoting a more aggressive tumor phenotype (Fig. 2) [76,77].

In a series of studies providing an explanation for the poor prognosis, including early tumor recurrence, lymph node metastasis, and tamoxifen-resistance, associated with stromal loss of caveolin-1 (Cav-1) in breast cancer [78], hydrogen peroxide secreted by cancer cells results in acute loss of caveolin-1 in TAFs, which then exhibit mitochondrial dysfunction, induction

of mitophagy for removal of damaged mitochondria, oxidative stress, and increased aerobic glycolysis (Fig. 2). Cancer cells, in turn, use the nutrients glycolytically supplied by TAFs to support their mitochondrial bioenergetics and suppress apoptosis. In non-nitric oxide synthase (eNOS)-expressing TAFs, Cav-1 was also down-regulated by neighboring eNOS-expressing fibroblasts, as both antioxidants and NO inhibitors reversed these effects [76]. As proof-of-principle, tamoxifen-sensitive and aerobic glycolysis-dependent MCF-7 cells become resistant to tamoxifen and reliant on the “reverse Warburg effect” when co-cultured with immortalized fibroblasts; however, combinatorial treatment with tamoxifen and dasatinib, which has a general antioxidant effect on both cancer cells and fibroblasts, returns MCF-7 cells to a “Warburg state” of aerobic glycolysis, making them unable to use the fuel supply from adjacent fibroblasts and rapidly killing them [77]. Thus, understanding the role of autophagy in oxidative stress management by TAFs may provide critical insight on how to best approach pharmacologic modulation of autophagy for reversing the “reverse Warburg effect” and overcoming treatment resistance.

5. Conclusions

Despite an early description of the autophagic process in the 1960s, only in recent years have its profound implications for disease pathogenesis been discovered. Autophagy is an evolutionarily conserved process that is critical for metabolic stress mitigation and preservation of cellular homeostasis by degrading damaged organelles and protein aggregates, in turn protecting cells from oxidative stress and maintaining genome integrity. Similar to other complex biological processes, many intracellular and environmental factors are at work to keep autophagy in balance.

The role of autophagy in cancer is dual-sided. Loss of autophagy may increase the propensity of cells toward oncogenic transformation, as autophagy-deficient cells are often more tumorigenic than their wild type counterparts in association with DNA damage accumulation, genomic instability and inflammation persistence. At the same time, autophagy is a major cell survival mechanism, as a catabolic process critical for ATP and amino acid recycling. This function is particularly important for cancer cells, which have high metabolic demands and a requirement for metabolic reprogramming, as evidenced by the Warburg and reverse Warburg effects. High rates of aerobic glycolysis and glutaminolysis lead to ROS production and oxidative stress in tumors, which commonly upregulate their own autophagy for survival. Cancer cell-induced oxidative stress may also stimulate autophagy in stromal cells and result in release of metabolites by TAFs and their subsequent utilization by neighboring cancer cells. Autophagy in cancer cells is also upregulated by hypoxia, a common occurrence in fast-growing tumors, and by potent oncogene activation, as evidenced by the dependence of mutant Ras-induced tumorigenesis on mitophagy and the preservation of a functional mitochondrial pool for cancer cell oxidative phosphorylation support.

Autophagy modulation has been proposed as both a cancer therapeutic and preventative measure [31,34,79]; however, the specifics of how to best accomplish these goals are still under intense investigation. Earlier work demonstrated that reconstitution of autophagy functional status diminishes the tumorigenic phenotype of autophagy-deficient cells [41], thus suggesting that autophagy stimulation could be a tumor suppressive strategy by counteracting organelle dysfunction and genomic instability. However, in the preclinical setting, the most successful way so far to modulate autophagy for cancer therapy has been concurrent, and thus acute, autophagy inhibition in combination with standard treatment regimens, mostly because autophagy is upregulated in cancer cells in response to anticancer agents as a prosurvival and treatment resistance mechanism. For example, autophagy inhibition sensitizes apoptosis-defective leukemic and colon cancer cells to TRAIL-

mediated apoptosis [80]; chloroquine, a non-specific autophagy inhibitor, enhances cyclophosphamide-induced tumor cell death in a murine Myc-induced lymphoma model [81]; 3-methyladenine, another non-specific autophagy inhibitor, in combination with 5-fluorouracil increased cancer cell apoptosis accompanied by tumor regression in colon cancer xenografts [82]. Concurrent modulation of cancer cell metabolism and autophagy has also been explored as an anticancer strategy, as human prostate cancer was treated with the combination of 2-deoxyglucose, which blocks glucose metabolism, and chloroquine [83]. Understanding the intertwined role of autophagy and cancer cell metabolism in tumor progression and its specific context-dependence will not only expand our understanding of cancer dynamics in different patients, but will also contribute to the identification of novel therapeutic targets for the development of personalized, and hopefully more effective, anticancer regimens.

Abbreviations

mTORC1	mammalian target of rapamycin complex 1
ULK1	Unc-51-like kinase complex
Atg	autophagy-related protein
TSC	tuberous sclerosis complex
AMP	adenosine monophosphate
AMPK	AMP kinase
HIF	hypoxia-inducible factor
MAP1LC3B or LC3	microtubule-associated protein 1 light chain 3 b
BNIP3	BCL2/adenovirus E1B protein-interacting protein
HCC	hepatocellular carcinoma
AT	alpha-1-antitrypsin
MHC-II	major histocompatibility complex class II
PDAC	pancreatic ductal adenocarcinoma
IFN	interferon
ROS	reactive oxygen species
ER	endoplasmic reticulum
TCA	tricarboxylic acid
TIGAR	TP53-induced glycolysis and apoptosis regulatory
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
CAFs	cancer-associated fibroblasts
Cav-1	caveolin-1
NOS	nitric oxide synthase
NO	nitric oxide

References

1. Mizushima N. Autophagy: process and function. *Genes & Development*. 2007; 21(22):2861–2873. [PubMed: 18006683]

2. Yang Z, Klionsky DJ. Eaten alive: a history of macroautophagy. *Nature Cell Biology*. 2010; 12(9): 814–822.
3. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature*. 2008; 451(7182):1069–1075. [PubMed: 18305538]
4. Rabinowitz JD, White E. Autophagy and metabolism. *Science*. 2010; 330(6009):1344–1348. [PubMed: 21127245]
5. Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. *Nature Reviews Cancer*. 2007; 7(12):961–967.
6. Mizushima N, Levine B. Autophagy in mammalian development and differentiation. *Nature Cell Biology*. 2010; 12(9):823–830.
7. Tsukamoto S, Kuma A, Mizushima N. The role of autophagy during the oocyte-to-embryo transition. *Autophagy*. 2008; 4(8):1076–1078. [PubMed: 18849666]
8. Tsukamoto S, Kuma A, Mizushima N. Autophagy is essential for preimplantation development of mouse embryos. *Science*. 2008; 321(5885):117–120. [PubMed: 18599786]
9. Cherra SJ 3rd, Dagda RK, Chu CT. Review: autophagy and neurodegeneration: survival at a cost? *Neuropathology and Applied Neurobiology*. 2010; 36(2):125–132. [PubMed: 20202120]
10. Garcia-Arencibia M, Hochfeld WE, Toh PP, Rubinsztein DC. Autophagy: a guardian against neurodegeneration. *Seminars in Cell & Developmental Biology*. 2010; 21(7):691–698. [PubMed: 20188203]
11. Neufeld TP. TOR-dependent control of autophagy: biting the hand that feeds. *Current Opinion in Cell Biology*. 2010; 22(2):157–168. [PubMed: 20006481]
12. He C, Klionsky DJ. Regulation mechanisms and signaling pathways of autophagy. *Annual Review of Genetics*. 2009; 43:67–93.
13. Thoreen CC, Sabatini DM. Rapamycin inhibits mTORC1: but not completely. *Autophagy*. 2009; 5(5):725–726. [PubMed: 19395872]
14. Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth: autophagy and metabolism. *Nature Cell Biology*. 2011; 13(9):1016–1023.
15. Takagi H, Matsui Y, Hirotsu S, Sakoda H, Asano T, Sadoshima J, et al. AMPK mediates autophagy during myocardial ischemia in vivo. *Autophagy*. 2007; 3(4):405–407. [PubMed: 17471015]
16. Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, et al. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science*. 2011; 331(6016):456–461. [PubMed: 21205641]
17. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *The Journal of Biological Chemistry*. 2008; 283(16):10892–10903. [PubMed: 18281291]
18. Schworer CM, Mortimore GE. Glucagon-induced autophagy and proteolysis in rat liver: mediation by selective deprivation of intracellular amino acids. *Proceedings of the National Academy of Sciences of the United States of America*. 1979; 76(7):3169–3173. [PubMed: 290994]
19. Gomes LC, Di Benedetto G, Scorrano L. Essential amino acids and glutamine regulate induction of mitochondrial elongation during autophagy. *Cell Cycle*. 2011; 10(16):2635–2639. [PubMed: 21811092]
20. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM, et al. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell*. 2010; 141(2):290–303. [PubMed: 20381137]
21. Mammucari C, Milan G, Romanello V, Masiero E, Rudolf R, Del Piccolo P, et al. FoxO3 controls autophagy in skeletal muscle in vivo. *Cell Metabolism*. 2007; 6(6):458–471. [PubMed: 18054315]
22. Sengupta A, Molkentin JD, Yutzev KE. FoxO transcription factors promote autophagy in cardiomyocytes. *The Journal of Biological Chemistry*. 2009; 284(41):28319–28331. [PubMed: 19696026]
23. Eng CH, Abraham RT. Glutaminolysis yields a metabolic by-product that stimulates autophagy. *Autophagy*. 2010; 6(7):968–970. [PubMed: 20724823]

24. Eng CH, Yu K, Lucas J, White E, Abraham RT. Ammonia derived from glutaminolysis is a diffusible regulator of autophagy. *Science Signaling*. 2010; 3(119):ra31. [PubMed: 20424262]
25. Sarkar S, Perlstein EO, Imarisio S, Pineau S, Cordenier A, Maglathlin RL, et al. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nature Chemical Biology*. 2007; 3(6):331–338.
26. Zhang L, Yu J, Pan H, Hu P, Hao Y, Cai W, et al. Small molecule regulators of autophagy identified by an image-based high-throughput screen. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(48):19023–19028. [PubMed: 18024584]
27. Qu X. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *Journal of Clinical Investigation*. 2003; 112(12):1809–1820. [PubMed: 14638851]
28. Yue Z, Jin S, Yang C, Levine AJ, Heintz N, et al. Beclin 1: an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(25):15077–15082. [PubMed: 14657337]
29. Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, et al. Autophagy promotes tumor cell survival and restricts necrosis: inflammation, and tumorigenesis. *Cancer Cell*. 2006; 10(1):51–64. [PubMed: 16843265]
30. Karantza-Wadsworth V, Patel S, Kravchuk O, Chen G, Mathew R, Jin S, et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. *Genes & Development*. 2007; 21(13):1621–1635. [PubMed: 17606641]
31. Yang ZJ, Chee CE, Huang S, Sinicrope FA. The role of autophagy in cancer: therapeutic implications. *Molecular Cancer Therapeutics*. 2011; 10(9):1533–1541. [PubMed: 21878654]
32. Wang K, Liu R, Li J, Mao J, Lei Y, Wu J, et al. Quercetin induces protective autophagy in gastric cancer cells: involvement of Akt-mTOR- and hypoxia-induced factor 1alpha-mediated signaling. *Autophagy*. 2011; 7(9):966–978. [PubMed: 21610320]
33. Vazquez-Martin A, Oliveras-Ferreros C, Menendez JA. Autophagy facilitates the development of breast cancer resistance to the anti-HER2 monoclonal antibody trastuzumab. *PLoS One*. 2009; 4(7):e6251. [PubMed: 19606230]
34. Chen N, Karantza V. Autophagy as a therapeutic target in cancer. *Cancer Biology & Therapy*. 2011; 11(2):157–168. [PubMed: 21228626]
35. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature*. 2006; 441(7095):885–889. [PubMed: 16625204]
36. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature*. 2006; 441(7095):880–884. [PubMed: 16625205]
37. Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, et al. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell*. 2007; 131(6):1149–1163. [PubMed: 18083104]
38. Singh R, Xiang Y, Wang Y, Baikati K, Cuervo AM, Luu YK, et al. Autophagy regulates adipose mass and differentiation in mice. *The Journal of Clinical Investigation*. 2009; 119(11):3329–3339. [PubMed: 19855132]
39. Zhang Y, Goldman S, Baerga R, Zhao Y, Komatsu M, Jin S, et al. Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106(47):19860–19865. [PubMed: 19910529]
40. Aita VM, Liang XH, Murty VV, Pincus DL, Yu W, Cayanis E, et al. Cloning and genomic organization of beclin 1: a candidate tumor suppressor gene on chromosome 17q21. *Genomics*. 1999; 59(1):59–65. [PubMed: 10395800]
41. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature*. 1999; 402(6762):672–676. [PubMed: 10604474]
42. Marino G, Salvador-Montoliu N, Fueyo A, Knecht E, Mizushima N, Lopez-Otin C, et al. Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/

- autophagin-3. *The Journal of Biological Chemistry*. 2007; 282(25):18573–18583. [PubMed: 17442669]
43. Takahashi Y, Coppola D, Matsushita N, Cualing HD, Sun M, Sato Y, et al. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nature Cell Biology*. 2007; 9(10):1142–1151.
 44. Mathew R, Kongara S, Beaudoin B, Karp CM, Bray K, Degenhardt K, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. *Genes & Development*. 2007; 21(11):1367–1381. [PubMed: 17510285]
 45. Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, et al. Autophagy-deficient mice develop multiple liver tumors. *Genes & Development*. 2011; 25(8):795–800. [PubMed: 21498569]
 46. O'Donovan PJ, Livingston DM. BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. *Carcinogenesis*. 2010; 31(6):961–967. [PubMed: 20400477]
 47. Scherz-Shouval R, Elazar Z. ROS: mitochondria and the regulation of autophagy. *Trends in Cell Biology*. 2007; 17(9):422–427. [PubMed: 17804237]
 48. Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, et al. Autophagy suppresses tumorigenesis through elimination of p62. *Cell*. 2009; 137(6):1062–1075. [PubMed: 19524509]
 49. Marciniak SJ, Lomas DA. Alpha1-antitrypsin deficiency and autophagy. *The New England Journal of Medicine*. 2010; 363(19):1863–1864. [PubMed: 21047232]
 50. Hidvegi T, Mukherjee A, Ewing M, Kemp C, Perlmutter DH. The role of autophagy in alpha-1-antitrypsin deficiency. *Methods in Enzymology*. 2011; 499:33–54. [PubMed: 21683248]
 51. Brest P, Corcelle EA, Cesaro A, Chargui A, Belaid A, Klionsky DJ, et al. Autophagy and Crohn's disease: at the crossroads of infection, inflammation, immunity, and cancer. *Current Molecular Medicine*. 2010; 10(5):486–502. [PubMed: 20540703]
 52. White E, Karp C, Strohecker AM, Guo Y, Mathew R. Role of autophagy in suppression of inflammation and cancer. *Current Opinion in Cell Biology*. 2010; 22(2):212–217. [PubMed: 20056400]
 53. Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature*. 2008; 456(7219):259–263. [PubMed: 18849966]
 54. Cadwell K, Patel KK, Komatsu M, Virgin HW, Stappenbeck TS. A common role for Atg16L1: Atg5 and Atg7 in small intestinal Paneth cells and Crohn disease. *Autophagy*. 2009; 5(2):250–252. [PubMed: 19139628]
 55. Li Y, Wang LX, Yang G, Hao F, Urba WJ, Hu HM, et al. Efficient cross-presentation depends on autophagy in tumor cells. *Cancer Research*. 2008; 68(17):6889–6895. [PubMed: 18757401]
 56. Pua HH, Dzhagalov I, Chuck M, Mizushima N, He YW. A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *The Journal of Experimental Medicine*. 2007; 204(1):25–31. [PubMed: 17190837]
 57. Pua HH, He YW, Dzhagalov I, Chuck M, Mizushima N, He YW, et al. Maintaining T lymphocyte homeostasis: another duty of autophagy. *Autophagy*. 2007; 3(3):266–267. [PubMed: 17329964]
 58. Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature*. 2008; 455(7211):396–400. [PubMed: 18701890]
 59. Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, et al. Pancreatic cancers require autophagy for tumor growth. *Genes & Development*. 2011; 25(7):717–729. [PubMed: 21406549]
 60. Wei H, Wei S, Gan B, Peng X, Zou W, Guan JL, et al. Suppression of autophagy by FIP200 deletion inhibits mammary tumorigenesis. *Genes & Development*. 2011; 25(14):1510–1527. [PubMed: 21764854]
 61. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metabolism*. 2008; 7(1):11–20. [PubMed: 18177721]

62. Flier JS, Mueckler MM, Usher P, Lodish HF. Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. *Science*. 1987; 235(4795):1492–1495. [PubMed: 3103217]
63. Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, et al. Akt stimulates aerobic glycolysis in cancer cells. *Cancer Research*. 2004; 64(11):3892–3899. [PubMed: 15172999]
64. Maiuri MC, Tasdemir E, Criollo A, Morselli E, Vicencio JM, Carnuccio R, et al. Control of autophagy by oncogenes and tumor suppressor genes. *Cell Death and Differentiation*. 2009; 16(1): 87–93. [PubMed: 18806760]
65. Lock R, Roy S, Kenific CM, Su JS, Salas E, Ronen SM, et al. Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. *Molecular Biology of the Cell*. 2011; 22(2):165–178. [PubMed: 21119005]
66. Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, et al. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes & Development*. 2011; 25(5):460–470. [PubMed: 21317241]
67. Kim MJ, Woo SJ, Yoon CH, Lee JS, An S, Choi YH, et al. Involvement of autophagy in oncogenic K-Ras-induced malignant cell transformation. *Journal of Biological Chemistry*. 2011; 286(15): 12924–12932. [PubMed: 21300795]
68. Bensaad K, Cheung EC, Vousden KH. Modulation of intracellular ROS levels by TIGAR controls autophagy. *The EMBO Journal*. 2009; 28(19):3015–3026. [PubMed: 19713938]
69. Yuneva M, Zamboni N, Oefner P, Sachidanandam R, Lazebnik Y. Deficiency in glutamine but not glucose induces MYC-dependent apoptosis in human cells. *The Journal of Cell Biology*. 2007; 178(1):93–105. [PubMed: 17606868]
70. Sakiyama T, Musch MW, Ropeleski MJ, Tsubouchi H, Chang EB. Glutamine increases autophagy under basal and stressed conditions in intestinal epithelial cells. *Gastroenterology*. 2009; 136(3): 924–932. [PubMed: 19121316]
71. Azad MB, Chen Y, Gibson SB. Regulation of autophagy by reactive oxygen species (ROS): implications for cancer progression and treatment. *Antioxidants & Redox Signaling*. 2009; 11(4): 777–790. [PubMed: 18828708]
72. Mazure NM, Pouyssegur J. Hypoxia-induced autophagy: cell death or cell survival? *Current Opinion in Cell Biology*. 2010; 22(2):177–180. [PubMed: 20022734]
73. Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, et al. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes & Development*. 2004; 18(23):2893–2904. [PubMed: 15545625]
74. Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkoul K, et al. The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *The Journal of Clinical Investigation*. 2010; 120(1): 127–141. [PubMed: 20038797]
75. Mbeunkui F, Johann DJ Jr. Cancer and the tumor microenvironment: a review of an essential relationship. *Cancer Chemotherapy and Pharmacology*. 2009; 63(4):571–582. [PubMed: 19083000]
76. Martinez-Outschoorn UE, Balliet RM, Rivadeneira DB, Chiavarina B, Pavlides S, Wang C, et al. Oxidative stress in cancer associated fibroblasts drives tumor-stroma co-evolution: a new paradigm for understanding tumor metabolism, the field effect and genomic instability in cancer cells. *Cell Cycle*. 2010; 9(16):3256–3276. [PubMed: 20814239]
77. Martinez-Outschoorn UE, Lin Z, Trimmer C, Flomenberg N, Wang C, Pavlides S, et al. Cancer cells metabolically fertilize the tumor microenvironment with hydrogen peroxide, driving the Warburg effect: implications for PET imaging of human tumors. *Cell Cycle*. 2011; 10(15):2504–2520. [PubMed: 21778829]
78. Bouras T, Lisanti MP, Pestell RG. Caveolin-1 in breast cancer. *Cancer Biology & Therapy*. 2004; 3(10):931–941. [PubMed: 15539932]
79. Hannigan AM, Gorski SM. Macroautophagy: the key ingredient to a healthy diet? *Autophagy*. 2009; 5(2):140–151. [PubMed: 19098459]

80. Han J, Hou W, Goldstein LA, Lu C, Stolz DB, Yin XM, et al. Involvement of protective autophagy in TRAIL resistance of apoptosis-defective tumor cells. *The Journal of Biological Chemistry*. 2008; 283(28):19665–19677. [PubMed: 18375389]
81. Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, et al. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *The Journal of Clinical Investigation*. 2007; 117(2):326–336. [PubMed: 17235397]
82. Li J, Hou N, Faried A, Tsutsumi S, Kuwano H. Inhibition of autophagy augments 5-fluorouracil chemotherapy in human colon cancer in vitro and in vivo model. *European Journal of Cancer*. 2010; 46(10):1900–1909. [PubMed: 20231086]
83. Stein M, Lin H, Jeyamohan C, Dvorzhinski D, Gounder M, Bray K, et al. Targeting tumor metabolism with 2-deoxyglucose in patients with castrate-resistant prostate cancer and advanced malignancies. *The Prostate*. 2010; 70(13):1388–1394. [PubMed: 20687211]

Fig. 1.

Autophagy and cancer cell metabolism. Diagram depicting the products of autophagy, their involvement in the Warburg effect, and the relationship between cancer cell metabolic reprogramming and its influence on autophagy. Autophagosomes containing damaged proteins and organelles are delivered to the lysosome for degradation via proteases, lipases, nucleases, etc. Carbohydrates are released and utilized by aerobic glycolysis and the pentose phosphate pathway (PPP) to produce energy, such as ATP, NADH, and NADPH, for cancer cell growth and proliferation. Nucleic acids are catabolized by the PPP and glycolysis, and along with carbohydrates, lipids and amino acids, are used for de novo biomolecular synthesis. Normally glycolytic products enter the tricarboxylic acid (TCA) cycle to maintain functional mitochondria; however, amino acids are redirected in cancer cell metabolism to processes such as glutaminolysis, which provides mitochondrial substrates to produce energy via the TCA cycle, NADPH for reducing harmful reactive oxygen species (ROS), and fatty acids. The process of autophagy is regulated by a variety of complex signals and extracellular factors. In the presence of cellular stress, ROS, and hypoxia, cells upregulate the process of autophagy for survival, however, when nutrients or growth factors are plentiful, cells inhibit the autophagic machinery to maintain homeostasis. Reprogrammed cancer cell metabolism and autophagy are intertwined in a continuous cycle, as autophagy

promotes cancer cell growth and proliferation by supporting its metabolism, altered cancer metabolism pathways stimulate autophagy. There is evidence that glycolytic enzymes, such as GAPDH, positively regulate autophagy genes and, therefore, indirectly upregulate autophagy. In a more direct fashion, glutaminolysis produces an ammonia byproduct, which is known to increase the autophagic process.

Fig. 2.

Tumor-stroma co-evolution and the Reverse Warburg effect. In this model, metabolic coupling occurs between tumor cells and tumor-associated fibroblasts (TAFs) as a result of oxidative stress in TAFs induced by neighboring tumor cells. Cancer progression-associated events, such as oncogene activation and recurrent hypoxia, result in tumor cells releasing reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), into their microenvironment. In turn, tumor-released ROS down-regulate caveolin-1 (Cav-1) in TAFs, aggravating oxidative stress and leading to further ROS production, this time by TAFs. In a vicious and self-perpetuating cycle, TAF-released ROS have a bystander effect on cancer cells, causing DNA damage and genomic instability, thus making tumors more aggressive. Furthermore, oxidative stress upregulates aerobic glycolysis and induces autophagy/mitophagy in TAFs resulting in the production of nutrients, such as lactate and ketones, which then stimulate mitochondrial biogenesis and oxidative metabolism in tumor cells (the reverse Warburg effect). Autophagy may play a role in Cav-1 down-regulation and damaged mitochondria removal, thus pushing TAFs toward aerobic glycolysis, as autophagy inhibitors readily reverse this effect.