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Molecular Pathways: Autophagy in Cancer—A Matter of Timing and Context

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Abstract

Autophagy is an intracellular self-digestion mechanism, by which cellular components are sorted into double-membrane autophagosomes and delivered to lysosomes for degradation. Cells utilize autophagy to dispose of wastes and eliminate hazards, while recycling nutrients and tuning metabolism in the process. Through these functions, autophagy promotes cell fitness, genome integrity, tissue homeostasis, and cell survival and growth under stress. Autophagy up- and down-regulation have both been found in human cancers, suggesting a complex role in tumor development. Accumulating results from autophagy-deficient mice and mouse models of human cancers have demonstrated that autophagy generally suppresses tumor initiation, but promotes tumor progression, in a manner that is dependent on timing and context and modified by specific tumorigenic events. Given the role of autophagy in facilitating tumor growth, autophagy inhibition has gained wide attention as a potential anticancer therapy. Here, we summarize relevant genetic, preclinical and clinical studies and discuss the multi-faceted role of autophagy in cancer, as well as the prospects of autophagy inhibition for cancer therapy.

Background

Autophagy as an intracellular self-digestion mechanism

Macroautophagy (autophagy hereafter) is a catabolic process whereby cellular material is enclosed in the double-membrane autophagosomes and delivered to lysosomes for degradation (1–4). Autophagy begins with the formation of a crescent-shaped phagophore (or isolation membrane) (Fig. 1). This highly regulated process involves 2 key kinases, the UNC-51-like kinase (ULK) and the Class III phosphatidylinositol 3-kinase VPS34, their associated regulatory factors, such as FIP200, Beclin-1, UVRAG and BIF-1, and several other autophagy-related (ATG) proteins (Fig. 1). Upon induction, ULK (ATG1) phosphorylates Beclin-1 (ATG6) and activates the VPS34 complex (5). VPS34 generates

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phosphatidylinositol 3,4,5-triphosphate (PI3P) on the membrane destined to become a phagophore, and PI3P recruits proteins required for phagophore elongation. Phagophore elongation requires the incorporation of phosphatidylethanolamine (PE)-lipidated LC3 (ATG8), whose formation is catalyzed by two “ubiquitin–like conjugation systems” composed of multiple other ATG proteins such as ATG5 and ATG7, etc. (2). The phagophore elongates until its membranes fuse, generating an autophagosome, which eventually fuses with the lysosome forming an autolysosome, where the resident lysosomal hydrolases breakdown the cargo.

Basal autophagy uses adaptor proteins, such as p62/SQSTM1 and NIX, to identify and deliver misfolded or aggregated proteins and damaged organelles to the autophagosome for degradation (6, 7), thereby preserving cellular fitness. Key to this selective cargo delivery are the specific interactions between the adaptor proteins and the phagophore membrane-bound LC3 (LC3-II), which serves as a cargo receptor Under stress conditions, such as oxygen and/or nutrient deprivation, autophagy is induced as a survival mechanism to recycle cytoplasmic constituents and generate fresh nutrients for cellular metabolism, e.g. macromolecule biosynthesis and energy production (8, 9). Stress-induced autophagy relies on non-selective engulfment of cytoplasmic material by the phagophore for degradation.

Autophagy suppresses oxidative stress and genome instability

Many studies have demonstrated the importance of functional autophagy in limiting oxidative stress (10–14). Electron leakage from mitochondrial electron transport to molecular oxygen is a main source of intracellular reactive oxygen species (ROS). As damaged mitochondria are mainly eliminated by autophagy (*i.e.* mitophagy) (15), autophagy-deficient cells accumulate dysfunctional mitochondria (12, 16), which are thought to be leaky and produce ROS. In addition, autophagy potentially may also reduce ROS levels by generating reducing powers through its metabolic function or by regulating proteins involved in antioxidant production.

Increased genome instability is a common feature of autophagy-deficient cells (10–12). This has been reviewed recently (17). Since ROS can cause mutations and DNA strand breaks, elevated ROS production in autophagy-deficient cells is considered as a key factor causing genome instability. Autophagy can also promote DNA repair and regulate cell division. During starvation, autophagy via its nutrient recycling function maintains the levels ATP and dNTP (8), both of which are important for DNA repair. Moreover, autophagy regulates specific proteins involved in lesion processing and nucleotide production, e.g. Sae2 and Rnr1 (18, 19). Finally, autophagy deficiency in yeast causes premature nuclear division in starvation, leading to aneuploidy upon nutrient replenishment (20).

Autophagy deregulation in human cancers

Autophagy was first linked to cancer when 40%, 50%, and 70% of prostate, breast, and ovarian cancers, respectively, were reported to have allelic loss of *BECN1*, which encodes the essential VPS34 complex component Beclin-1 (21). This finding suggests that Beclin-1, and autophagy in general, may be a tumor suppressor. However, recent investigation of *BECN1* mutational status in The Cancer Genome Atlas (TCGA) has called into question the

significance of allelic *BECN1* deletions in cancers (22), since they occur in conjunction with deletion of the breast and ovarian tumor suppressor gene *BRCA1*, located only ~150 KB away on chromosome 17q21. Notably, hypermethylation of the *BECN1* promoter has been observed in up to 70% of breast cancers (23), and low *BECN1* mRNA levels have been reported in ovarian (24), colon (25), brain (26), and liver (27) malignancies. Thus, defective autophagy due to epigenetic silencing and/or transcriptional inhibition of *BECN1* may play a role in the etiology and/or progression of these tumors.

Other than *BECN1*, allelic *UVRAG* loss is noted in colon (28) and gastric (29) cancers; whereas LAPTM4B, which maintains lysosomal pH and allows autophagosome-lysosome fusion, is frequently amplified and overexpressed in breast cancer (30). Furthermore, cancers often exhibit functional suppression of autophagy, especially upon activation of the PI3K-AKT-mTOR pathway, which inhibits autophagy (31). For example, approximately 25% of breast cancers have amplification of *HER2* (*ERBB2*), which encodes a receptor tyrosine kinase that activates the above pathway.

Autophagy-deficient mice and allograft/xenograft studies

Homozygous *Becn1* deletion leads to embryonic lethality, while *Becn1*$^{+/-}$ mice are viable, display partial autophagy defect and develop liver and lung tumors, lymphomas, and mammary hyperplasias (32, 33). Monoallelic *Becn1* loss-associated mammary hyperplasias progress to tumors following parity in FVB background, but not in C57BL/6 background (34). Mice with mosaic *Atg5* deletion or liver-specific *Atg7* deletion develop benign hepatomas (35), and *Bif-1* knockout mice show increased incidence of lymphomas (36). Collectively, these results indicate that basal autophagy suppresses tumor development. Consistent with this notion, *Becn1*$^{+/-}$ immortalized mouse mammary epithelial cells (iMMECs) and baby mouse kidney (iBMK) cells are more tumorigenic than their *Becn1*$^{+/+}$ counterparts in nude mice (10, 13). Also, EGFR- and AKT-mediated Beclin-1 phosphorylation, which both attenuate autophagy, promote the growth of lung and breast cancer xenografts, respectively (37, 38).

Genetic modulation of autophagy in mouse tumor models

Complete or partial autophagy deficiency has been introduced into several mouse tumor models (Table 1). As aging *Becn1*$^{+/-}$ mice develop spontaneous lymphomas (32, 33), the role of autophagy has been assessed in mouse lymphoma models. In *Atm*$^{-/-}$ mice, which spontaneously develop lymphomas, monoallelic *Becn1* loss delayed tumor development and increased survival (T_{50}=262 vs 137 days, p=0.006) (39). In contrast, monoallelic *Becn1* loss in an Eμ-MYC-driven lymphoma model led to faster tumor development and decreased survival (T_{50}=80 vs 142 days, p=0.007) (39). *Bif-1*$^{-/-}$ and *Bif-1*$^{+/-}$ mice also showed accelerated Eμ-MYC-driven lymphomagenesis (T_{50}=65 and 75 days vs 107 days, p=0.0006 and p<0.0001, respectively) (12). These studies demonstrate that the role of autophagy in lymphoma depends on the specific drivers of tumor development.

The role of autophagy has also been assessed in multiple breast cancer models. In the polyoma middle T-antigen (MMTV-PyMT) driven model, conditional biallelic *Fip200* deletion delayed tumor development (T_{50}=85 vs 62 days, p<0.01) (40). In a model of

hereditary breast cancer, monoallelic *Becn1* loss delayed and reduced tumor development induced by conditional loss of the PALB2 tumor suppressor (27% vs 66% penetrance, p=0.0035) (41). However, *Becn1* heterozygosity did not affect mammary tumorigenesis driven by HER2 or PyMT oncogenes (42). Interestingly, monoallelic *Becn1* loss accelerated WNT1-driven mammary tumorigenesis (T_{50}=120 vs 219 days, p=0.004) (34). In this case, allelic *BECN1* loss appears to promote mammary tumorigenesis by deregulating the mammary hierarchy and expanding the mammary progenitor cell (MaPC) population, thus cooperating with WNT1 activation, which drives MaPC transformation.

Recent studies using the $KRAS^{G12D}$- and $BRAF^{V600E}$-driven models of non-small cell lung cancer (NSCLC) have revealed that autophagy defects both promote early tumorigenesis (43, 44) and impede tumor progression (44, 45). Conditional biallelic *Atg7* deletion slowed the progression of $KRAS^{G12D}$–driven lung tumors, but was not associated with any survival benefit, as mice with *Atg7*-deficient pulmonary epithelium developed pneumonia and died at the same time as the control mice with higher tumor burden (45). Interestingly, *Atg7* deletion in a $BRAF^{V600E}$-driven NSCLC mouse model accelerated the onset of tumor development but delayed tumor progression and prolonged survival (44). The same dual effects were also observed when *Atg5* was deleted in the $KRAS^{G12D}$-driven lung cancer model (43). An important finding from these studies is that autophagy impacts the histological fate, and likely the aggressiveness, of $KRAS^{G12D}$- and $BRAF^{V600E}$-driven lung tumors, as either *Atg5* or *Atg7* deletion results in development of oncocytomas instead of adenocarcinomas (43–45), which are benign tumors characterized by massive accumulation of abnormal mitochondria (46).

RAS-mutant pancreatic cancer cells have high basal autophagy and seem to be “addicted” to autophagy for growth (47). Recently, the role of autophagy in pancreatic ductal adenocarcinoma (PDAC) has been thoroughly assessed in a $KRAS^{G12D}$-driven model in two separate studies (48, 49). Normally, these mice develop a small number of pre-malignant pancreatic intraepithelial neoplasia (PanIN) lesions, which stochastically evolve into PDAC over time. Interestingly, conditional biallelic deletion of either *Atg5* or *Atg7* led to increased incidence of PanIN, but blocked its progression to high-grade hyperplasia and PDAC (48, 49), reminiscent of the effect produced by *Atg7* or *Atg5* deletion in the above BRAF- and KRAS-driven lung cancer models, respectively.

Besides the timing-, context- and dosage-dependent role of autophagy in tumor development, the above studies also reveal p53 status as a key parameter impacting the role of autophagy in cancer. Specifically, *Becn1* haploinsufficiency delayed *Palb2*-associated mammary tumorigenesis in *Trp53*-wild type mice, but not when *Trp53* was co-deleted with *Palb2* in the mammary epithelium (41). Similarly, co-deletion of *Trp53* diminished or eliminated the inhibitory effect of either *Atg5* or *Atg7* deletion on tumor progression in both KRAS- and BRAF-driven lung cancer models (43–45). These results indicate that a key function of autophagy may be to limit p53 induction and/or overcome the barrier imposed by p53 activation to tumorigenesis, and they also suggest that autophagy may be less relevant in p53-mutant cancers. However, in the $KRAS^{G12D}$-driven PDAC model, *Atg5* or *Atg7* ablation accelerated tumor progression and reduced survival when *Trp53* was also deleted simultaneously (48). Thus, how p53 status influences the role of autophagy in cancer

may vary from tissue to tissue. A possible caveat of the above models is that they all involve biallelic *Trp53* deletion at the time of another oncogenic event, whereas in human cancers, *TP53* status generally changes by acquisition of point mutations and loss of heterozygosity (LOH) over time. Nonetheless, given its key role in cell cycle control, apoptosis and metabolism, the final *TP53* status in established tumors is still likely to modify the impact of autophagy defect or inhibition on tumor growth and progression.

Finally, studies using tumor-derived cell lines from the above models have revealed novel functions of autophagy in metabolism. In particular, *Atg7*–null KRAS- and BRAF-driven lung cancer cells showed defects in mitochondrial lipid oxidation and low levels of tricarboxylic acid (TCA) cycle intermediates and glutamine, which was found to be critical for the survival of the cancer cells (44, 45, 50). Also, decreased glycolysis was observed in *Fip200*-deficient mammary tumor cells (40), potentially connecting autophagy to increased aerobic glycolysis in cancer, known as the “Warburg effect”.

Clinical-Translational Advances

Chemical autophagy inhibition *in vitro* and in mouse tumor models

Autophagy inhibitors can be broadly divided into two groups, with one group inhibiting autophagosome formation (early-stage inhibitors) and the other blocking autophagosome-lysosome fusion (late-stage inhibitors). The currently available early-stage inhibitors, including 3-Methyladenine (3-MA), wortmannin, LY294002 and the newly identified spautin-1, all target the VPS34 complex. The first three interfere with its membrane recruitment (51), whereas spautin-1 promotes its degradation (52). Late-stage inhibitors include bafilomycin A1, chloroquine (CQ) and hydroxychloroquine (HCQ). These drugs effectively inhibit lysosome acidification, block autophagosome-lysosome fusion and, thus, cargo degradation (51).

The efficacy of pharmacologic autophagy inhibition in killing cancer cells has been assessed in numerous studies. As single agents, autophagy inhibitors generally inhibit the growth and/or survival of cancer cells with high basal autophagy, such as RAS-driven cancer cells, which markedly upregulate and are addicted to autophagy (47, 50). CQ/HCQ treatment also delays tumorigenesis and/or improved survival in lymphomas caused by either *Atm* deficiency or MYC activation (53, 54). Importantly, dozens of anticancer agents as well as radiation have been found to induce autophagy, and cancer cells not relying on autophagy under normal growth conditions may induce autophagy as a survival mechanism in response to anticancer therapies (55–57). Thus, combining autophagy inhibition with autophagy-inducing therapeutics may achieve better tumor cell killing. For example, CQ sensitized HER2-positive and hormone-refractory estrogen receptor (ER)-positive breast cancer cells to HER2-targeting therapies (42) and tamoxifen (58), respectively. This strategy has seen promise in pre-clinical studies and is currently under clinical investigation. However, it should be noted that there exists evidence that suggests that therapy-induced autophagy may contribute to cell killing (56, 57). Thus, the role of autophagy in cancer therapy needs to be determined case by case in different cancer-drug combinations.

Clinical trials involving autophagy modulation by HCQ

There are currently 27 NCI-registered clinical trials actively evaluating the therapeutic efficacy of pharmacologic autophagy inhibition in different tumor types. In all trials, autophagy is modulated by HCQ given in combination with standard chemotherapy (and concurrent radiation therapy in one trial). In an earlier Phase I study, addition of HCQ to erlotinib produced partial response (PR) in 1 out of 19 NSCLC patients with EGFR-mutant tumors (59). The responding patient received 600 mg HCQ daily. Results of several other studies have recently become available. In a phase I/II trial of HCQ in conjunction with radiation therapy and concurrent/adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme (GBM), the maximum tolerated dose (MTD) for HCQ was 600 mg daily, which resulted in inconsistent autophagy inhibition, as evaluated by autophagic vacuole (AV) formation in peripheral blood mononuclear cells (PBMCs), and did not prolong overall survival (60). In a phase I study combining the HDAC inhibitor vorinistat with escalating doses of HCQ in patients with advanced solid tumors, the MTD for HCQ was again 600 mg daily; although treatment-related changes in AV numbers in PBMCs were not seen, increases in the expression of CDKN1A and CTSD were reported and were more pronounced in tumor biopsies than PBMCs; out of 24 evaluable patients, 1 patient with renal cell carcinoma (RCC) had confirmed durable PR and 2 patients with colorectal cancer have prolonged stable disease (SD) (61). In a phase I trial combining bortezomib and HCQ for relapsed/refractory myeloma, PR, minor response and SD were observed in 14%, 14% and 45% of patients, respectively (62). In this study, HCQ was given at 600 mg twice daily with standard doses of bortezomib and resulted in therapy-associated AV number increases in bone marrow plasma cells. In another phase I study, combination of HCQ with dose-intense temozolomide resulted in 14% PR and 27% SD in patients with metastatic melanoma and induced significant AV accumulation in PBMCs; the recommended phase II dose was HCQ 600 mg twice daily (63). Finally, combined MTOR and autophagy inhibition in a phase I/II trial of HCQ and temsirolimus resulted in SD in about 70% of patients with melanoma; the HCQ dose in the phase II part of the study was 600 mg twice daily and at that, but not lower, dose, autophagy inhibition was documented in PMBCs and tumor biopsies (64).

Taken together, the results of the above studies indicate that, when tolerated, the combination of HCQ at higher dose (600 mg twice daily) with standard chemotherapy regimens modulates autophagy in patients and has antitumor activity. Treatment-related toxicities limit the use of high-dose HCQ in combination with vorinostat and during adjuvant chemoradiotherapy for newly resected GBM, thus indicating a need for the development of lower toxicity compounds that inhibit autophagy more consistently than HCQ.

CONCLUSIONS AND PERSPECTIVES

The role of autophagy in cancer is complex and varies depending on the timing and context of tumor development. Yet, despite the seemingly paradoxical roles of autophagy under different settings, two principles have been emerging from the mouse models studied so far. First, basal autophagy generally suppresses tumor initiation. This may be achieved by suppressing ROS, which causes DNA damage and genome instability, thereby promoting

age-associated, spontaneous tumor development, as seen in autophagy deficient mice. Also, modestly increased ROS can directly stimulate cell growth, which may contribute to the initial acceleration of tumor (or pre-cancerous lesion) formation observed in the above oncogene-driven models. Second, in contrast to its suppressive role in tumor initiation, autophagy facilitates tumor progression in most cases examined so far. This function may be exerted by autophagy-mediated mitigation of excessive ROS, suppression of DNA damage response, recycling of nutrients for biosynthesis and energy production, maintenance of mitochondrial and lipid metabolism, and possibly degradation of key regulators of cell growth.

How the functional status of autophagy impacts tumorigenesis likely depends on how ROS levels, DNA damage and autophagy-related metabolism affect the fate of a given tumor at a particular stage of its trajectory. The outcome may be decided by the specific oncogene activation and/or tumor suppressor loss that drives cancer initiation and progression, perhaps in conjunction with the tissue/cell type of origin. It is important to appreciate the genetic/epigenetic and physiological differences between early and late stage tumors. Early lesions tend to be more “authentic” and uniform, therefore may be affected more readily by changes in autophagy status. Late stage tumors have often acquired additional alterations in key regulatory genes, such as *TP53* and *NRF2*, which may dramatically alter their requirement for autophagy and therefore its role in tumor progression.

Given the role of autophagy in generally facilitating tumor progression, targeting autophagy has considerable potential for cancer therapy. To better harness this potential, it is critical to determine the exact “context” for each tumor, particularly the status of key oncogenes and tumor suppressors, such as the ones noted before. As tumors with high levels of basal autophagy, ROS and DNA damage or under considerable metabolic stress are more likely reliant on autophagy for survival and growth, and should thus be more sensitive to drugs that inhibit autophagy, a set of effective biomarkers to diagnose these autophagy-addicted malignancies may be useful. Also, given the context-dependent tumor-suppressive function of autophagy, the long-term consequences of chronic or periodic pharmacologic autophagy inhibition need to be determined. In the same vein, it is imperative to develop novel agents that specifically target mechanisms upregulating autophagy in cancer cells, while leaving basal autophagy in normal cells intact.

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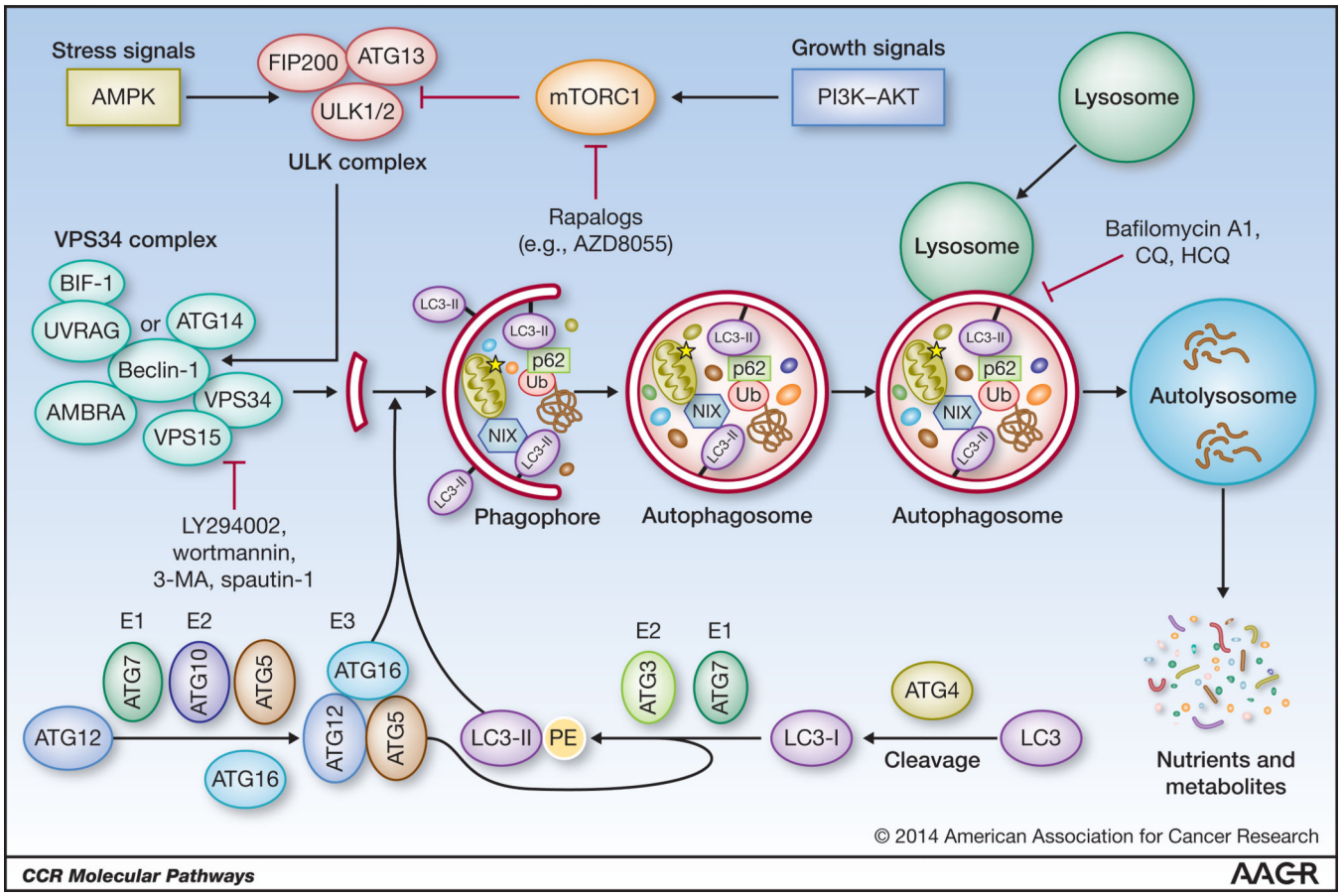

Figure 1.

The autophagic process. Upon deprivation of nutrients or growth factors, activation of AMPK and/or inhibition of mTOR lead to activation of ULK, which phosphorylates Beclin-1, leading to VPS34 activation and phagophore formation. ULK functions in a complex with FIP200 and ATG13, while VPS34 function requires a regulatory subunit, VPS15 (p150), and Beclin-1, which further mediates the association of other regulatory factors such as AMBRA, ATG14, UVRAG and BIF-1. Multiple ATG proteins such as ATG5 and ATG7 constitute two “ubiquitin–like conjugation systems” that catalyze the formation of phosphatidylethanolamine (PE)-conjugated LC3 (LC3-II) and direct its proper incorporation into the phagophore membrane, where it serves as docking site of adaptor proteins (and bound cargos). The closure of an elongated phagophore marks the formation of a mature autophagosome, which eventually fuses with a lysosome, leading to cargo degradation and recycling of nutrients and metabolites. Ub, ubiquitin.

Table 1

Tumor phenotypes of autophagy-deficient mice and the effect of autophagy deficiency on tumor development in established mouse tumor models.

Autophagy defect	Oncogenic driver	Genetic background	Lesion or tumor type	Effect	Ref.
Becn1$^{+/-}$	N/A	C57BL/6	Liver/lung tumors, lymphomas, mammary hyperplasias (independent of parity)	↑ ↑	32, 33
Becn1$^{+/-}$	N/A	FVB/N	Mammary hyperplasias in nulliparous mice Mammary tumors after parity	↑ ↑	34
Atg5$^{F/F}$;CAG-*Cre*	N/A	C57BL/6	Hepatomas	↑	35
Mosaic *Atg7* deletion	N/A	C57BL/6	Hepatomas	↑	35
Bif1$^{-/-}$	N/A	mixed	Lymphomas	↑	36
Becn1$^{+/-}$	*Atm*$^{-/-}$	C57BL/6	Lymphoma	↓	39
Becn1$^{+/-}$	Eu-Myc	C57BL/6	Lymphoma	↑	39
Bif1$^{-/-}$ and *Bif1*$^{+/-}$	Eu-Myc	C57BL/6	Lymphoma	↑	12
FIP200$^{F/F}$;MMTV-*Cre*	MMTV-*PyMT*	FVB/N	Mammary tumors Lung metastases	↓ ↓	40
Becn1$^{+/-}$	*Palb2*$^{F/F}$;WAP-*Cre*	Mixed	Mammary tumors	↓	41
Becn1$^{+/-}$	*Palb2*$^{F/F}$;*Trp5*$^{F/F}$;WAP-*Cre*	Mixed	Mammary tumors	Neutral	41
Becn1$^{+/-}$	MMTV-*Neu*	Mixed	Mammary tumors	Neutral	42
Becn1$^{+/-}$	MMTV-*PyMT*	FVB/N	Mammary tumors	Neutral	42
Becn1$^{+/-}$	MMTV-*Wnt1*	FVB/N	Mammary tumors (basal-like)	↑	34
Atg5$^{F/F}$;Adeno-*Cre**	LSL-*Kras*(G12D)	ND	Early lung tumors Late lung tumors***	↑ ↓	43

Autophagy defect	Oncogenic driver	Genetic background	Lesion or tumor type	Effect	Ref.
Atg5$^{F/F}$;Adeno-*Cre**	LSL-*Kras*(G12D);*Trp53*$^{F/F}$**	ND	Early lung tumors Late lung tumors***	Neutral Neutral	43
Atg7$^{F/F}$;Adeno-*Cre**	LSL-*Kras*(G12D)	C57BL/6	Early lung tumors Late lung tumors***	ND ↓	45
Atg7$^{F/F}$;Adeno-*Cre**	LSL-*Kras*(G12D);*Trp53*$^{F/F}$**	ND	Early lung tumors Late lung tumors***	ND ↓	45
Atg7$^{F/F}$;Adeno-*Cre**	LSL-*Braf*(V600E)	mixed	Early lung tumors Late lung tumors***	↑ ↓	44
Atg7$^{F/F}$;Adeno-*Cre**	LSL-*Braf*(V600E);*Trp53*$^{F/F}$**	mixed	Early lung tumors Late lung tumors***	↑ ↓	44
Atg5$^{F/F}$;Pdx1-*Cre*	LSL-*Kras*(G12D)	ND	Low-grade PanIN High-grade PanIN and PDAC	↑ ↓	48
Atg7$^{F/F}$;Pdx1-*Cre*	LSL-*Kras*(G12D)	ND	Low-grade PanIN High-grade PanIN and PDAC	↑ ↓	48
Atg5$^{F/F}$;Pdx1-*Cre*	LSL-*Kras*(G12D);*Trp53*$^{F/F}$**	ND	PDAC	↑	48
Atg7$^{F/F}$;Pdx1-*Cre*	LSL-*Kras*(G12D);*Trp53*$^{F/F}$**	ND	PDAC	↑	48
Atg5$^{F/F}$;Pdx1-*Cre*	LSL-*Kras*(G12D);*Trp53*$^{F/W}$	mixed	Low-grade PanIN High-grade PanIN and PDAC	↑ ↓	49

ND, not described;

* deletion is driven by intranasal delivery of Adeno-Cre;

** *Trp53* is co-deleted with *Atg5* or *Atg7* in the same cells;

*** in autophagy-proficient setting, lung tumors (early and late) are adenomas and adenocarcinomas under *Trp53*$^{+/+}$ and *Trp53*-null conditions, respectively, whereas in autophagy-deficient setting, late lung tumors are benign oncocytomas regardless of *Trp53* status.