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Review

Recent Advances in the Role of Autophagy in Endocrine-Dependent Tumors

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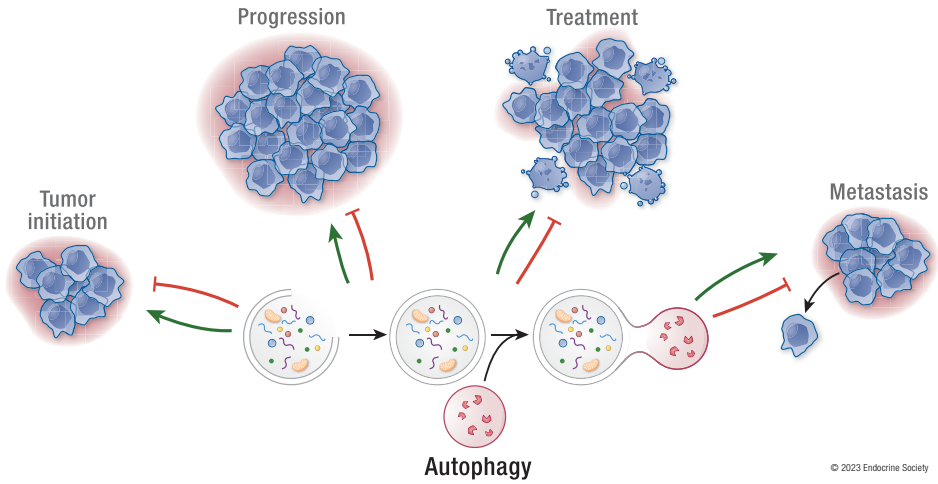
Abstract

Autophagy plays a complex role in several cancer types, including endocrine-dependent cancers, by fueling cellular metabolism and clearing damaged substrates. This conserved recycling process has a dual function across tumor types where it can be tumor suppressive at early stages but tumor promotional in established disease. This review highlights the controversial roles of autophagy in endocrine-dependent tumors regarding cancer initiation, tumorigenesis, metastasis, and treatment response. We summarize clinical trial results thus far and highlight the need for additional mechanistic, preclinical, and clinical studies in endocrine-dependent tumors, particularly in breast cancer and prostate cancer.

Graphical Abstract



Key Words: autophagy, cancer, endocrine-Dependent tumors, chloroquine, clinical trials

Abbreviations: AMBRA1, Autophagy And Beclin 1 Regulator 1; ATG, autophagy related; *BECN1*, Beclin 1; CQ, chloroquine; EMT, epithelial to mesenchymal transition; ER, endoplasmic reticulum; GABARAP, Gamma-aminobutyric acid receptor-associated protein; HCQ, hydroxychloroquine; HER2, Human Epidermal Growth Factor Receptor 2; LC3, Light chain 3; mTOR, mechanistic target of rapamycin; NPRL2, Nitrogen permease regulator-like 2; PDAC, Pancreatic ductal adenocarcinoma; Pfkfb3, 6-Phosphofructo-2-kinase/fructose 2, 6-biphosphatase 3; REST, repressor element-1 silencing transcription factor; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptors; TME, tumor microenvironment; TNC, Tenascin C; ULK, Unc-51–like kinase; UVRAG, UV radiation resistance-associated gene protein.

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ESSENTIAL POINTS

- Autophagy plays a complex role in several cancer types, including endocrine-dependent cancers, by fueling cellular metabolism and clearing damaged substrates
- The review provides an overview of the autophagic pathway and its roles in cancer
- We focus on the dual nature of autophagy across tumor types where it can be tumor suppressive at early stages but tumor promotional in established disease
- The review highlights the roles of autophagy in endocrine-dependent tumors regarding cancer initiation, tumorigenesis, metastasis, and treatment response
- We provide insight into when autophagy modulation might benefit patients with endocrine-dependent malignancies and summarize clinical trial results thus far

Autophagy is a catabolic process that degrades a variety of cytoplasmic material via lysosomes (1, 2). Over the last decade, studies have revealed a crucial role for autophagy in several cancer types—including endocrine-dependent cancers—by fueling cellular metabolism and clearing damaged substrates. Historically, it was first observed that the core autophagy gene Beclin 1 (*BECN1*) is lost in many cancer types, suggesting a tumor suppressive role for the recycling process (3, 4). A couple of decades later, however, autophagy is now increasingly recognized as playing a dual role: while it appears to limit tumor initiation, numerous studies have reported that autophagy promotes the growth of already established tumors by providing essential resources to cancer cells even in harsh environments (5). In addition, a growing body of literature supports the use of autophagy inhibitors as a means to improve the efficiency of conventional cancer treatments (5, 6). Based on these observations, autophagy inhibitors are currently underway or are moving forward in clinical trials in several tumor types, including endocrine-dependent tumors such as breast cancer (NCT00765765, NCT01023477, NCT03774472, NCT04316169, NCT04523857, NCT04841148, NCT03032406) and prostate cancer (NCT02421575, NCT00786682, NCT05036226, NCT00726596, NCT01480154, NCT01828476, NCT03513211, NCT04011410).

Here, we will provide an overview of the autophagic pathway and its roles in cancer. The goal of this review is to highlight the roles of autophagy in endocrine-dependent tumors regarding cancer initiation, tumorigenesis, metastasis, and treatment response. Autophagy has been heavily studied across many types of cancer. In order to provide perspective on how to best target autophagy in endocrine-related tumors, this review will also contextualize these findings amongst the broader field of autophagy across different tumor types.

Autophagy

Autophagy is an evolutionarily conserved recycling processes that facilitates the delivery of excess or damaged cytoplasmic material to lysosomes for degradation. It is fundamental for protein turnover, organelle quality control, and cellular metabolism as well as innate and adaptive immunity (7). It has also been implicated in numerous pathologies including neurodegenerative diseases, metabolic disorders, and cancer (8). To date, 3 distinct types of autophagy have been identified: microautophagy, chaperone-mediated autophagy, and macroautophagy—all of which eventually lead to the digestion of cargo by lysosomal enzymes. The byproducts are subsequently released back into the cytosol and recycled as new building blocks (2). This review will focus on macroautophagy, hereafter referred to as autophagy. Microautophagy—whereby cargos are engulfed directly via invagination of the lysosomal membrane—and chaperone-mediated autophagy—which relies on the coordination of chaperones and a protein translocation system—have been reviewed in detail elsewhere (2, 9, 10).

Macroautophagy

Autophagy is a tightly regulated process involving over 20 core proteins (11–13). Upstream activation of the pathway is regulated by cell energy balance and nutrient availability. Major upstream players include the nutrient sensors AMP kinase (AMPK) and the mechanistic target of rapamycin (mTOR) kinase, which stimulate and repress autophagy, respectively (14, 15). Autophagy can be triggered by a variety of stimuli including hypoxia, oxidative stress, DNA damage, protein aggregates, dysfunctional organelles, or infection by pathogens (1, 7). It can be integrated as part of a coordinated stress response along with the activation of other pathways including metabolism, and cell cycle and cell growth processes (16).

Autophagy is a complex multistep process that involves the formation of a double membrane vesicle, known as an autophagosome, in order to degrade dysfunctional or excess proteins and organelles. The recycled cytoplasmic material is broken down into macromolecular building blocks that can be used to fuel diverse metabolic pathways (17, 18). A host of core autophagy-related (ATG) proteins are necessary to facilitate this multistep pathway, which includes (1) vesicle initiation, (2) nucleation and the formation of a double membrane structure, (3) vesicle elongation, and finally (4) fusion with lysosomes and ultimate degradation (Fig. 1) (12, 17, 19–22).

The early steps in autophagy are mediated by the Unc-51–like kinase (ULK) complex comprising ULK1, ULK2, ATG13, FAK family kinase-interacting protein of 200 kDa (FIP200/RB1CC1), and ATG101. Under nutrient replete conditions, mTOR complex 1 (mTORC1) phosphorylates the transcription factor EB. Following autophagy induction and mTOR inhibition, transcription factor EB is dephosphorylated and translocates from the cytosol to the nucleus, allowing the transcription of key lysosomal and autophagy genes (23–25). In addition, mTOR inhibition also leads to the dephosphorylation of ULK1 and ATG13, thereby activating the ULK complex via ULK1 autophosphorylation (26–29). The ULK complex then translocates to autophagy initiation sites where it activates the complex formed by BECN1 with phosphatidylinositol 3-kinase catalytic subunit type 3, the mammalian homolog of yeast Vps34 (PIK3C3/VPS34), UV radiation resistance-associated gene protein (UVRAG), activating molecule in BECN1-regulated autophagy protein 1 (AMBRA1), and ATG14 (30–35). This leads to the generation of a local pool of phosphatidylinositol-3-phosphate on forming autophagosome membranes, known as phagophores. The

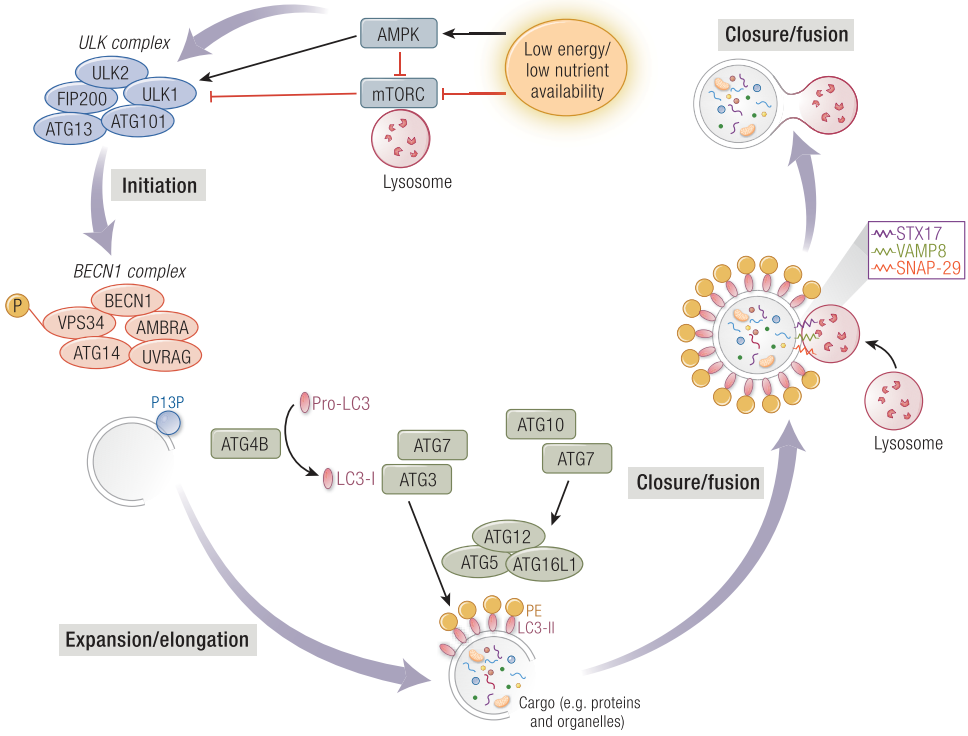

Figure 1. Overview of the autophagy pathway. The autophagy pathway comprises of 5 main stages: initiation, expansion, elongation, closure, and fusion. Autophagy is tightly regulated by the nutrient sensors mTOR and AMPK which can inhibit or activate the ULK1 complex to trigger autophagy. The ULK1 complex then activates the Beclin 1 complex that triggers the formation of autophagosomes. Several ATG proteins then help convert LC3 to its active form and incorporate into forming autophagosomes that engulf cargo that has been tagged for degradation by cargo receptors. Once the autophagosome has formed, SNARE proteins such as STX17, VAMP8, and SNAP-29, enable the fusion of autophagosomes with lysosomes. Lysosomal enzymes then degrade the cargo to form metabolites that can be used for downstream reactions.

origin of the phagophore membrane remains incompletely understood but models have postulated its generation from multiple sources including endosomal and Golgi vesicles, mitochondrial membranes, and the endoplasmic reticulum (ER) (18, 36–42). The BECN1 complex promotes both formation and extension of the phagophore membrane.

Subsequently, 2 distinct ubiquitin-like conjugation systems facilitate phagophore elongation. The first reaction requires the E1-like enzyme, ATG7, and the E2-like enzyme, ATG10, which together catalyze the conjugation of ATG5 and ATG12. The ATG5-12 conjugate goes on to form a multiprotein complex with ATG16L1. The second ubiquitin-like conjugation reaction relies on the ATG5–ATG12–ATG16L1 complex to function as the E3 enzyme, with ATG7 acting again as the E1 and ATG3 as E2, to conjugate phosphatidylethanolamine to Gamma-aminobutyric acid receptor-associated protein (GABARAP)/Light chain 3 (LC3) (17, 43, 44). There are several GABARAP proteins (also known as Atg8 in yeast); the most well characterized is LC3B (45). Prior to lipidation, pro-LC3 must first be cleaved by the family of cysteine proteases, including ATG4B, to create the conjugation-ready LC3-I molecule. The functional LC3–phosphatidylethanolamine conjugate also known as LC3-II associates with the growing autophagosome membrane (20). LC3-II and all the conjugation machinery are essential for efficient autophagosome formation and closure as well as subsequent fusion with lysosomes (45–47). Because LC3-II is embedded in the membrane it is both necessary for autophagosome turn over but also itself degraded in the process.

The closure of the phagophore results in the formation of a completed autophagosome, which when fused to a lysosome, forms an autolysosome. This is enabled by the soluble N-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) complex. In mammals, Qa-SNARE syntaxin 17 (STX17) is the major SNARE protein. It recruits SNARE Synaptosomal-associated protein 29 (SNAP-29) and R-SNARE Vesicle Associated Membrane Protein 8 (VAMP8) to form a complex which mediates autophagosome–lysosome fusion (48, 49). Acidic lysosomal hydrolases then break down the autophagic substrates into macromolecular building blocks such as amino acids that are recycled back into the cytoplasm and reused to fuel metabolic pathways.

Selective Autophagy

In response to starvation, mTORC1 inhibition promotes non-selective or bulk autophagy, which involves the sequestration of bulk portions of the cytoplasm into phagophores as a means to maintain appropriate intracellular amino acid and nutrient levels (2, 7, 15). In contrast, selective autophagy is used for quality control by targeting specific, often damaged, cargos while preserving the bulk cytoplasm (7, 50). Selective autophagy is also important in protecting the cell from invasive pathogens (51, 52). The most well-characterized forms of selective autophagy target mitochondria (mitophagy) (53–55), ER (ER-phagy) (56, 57), peroxisomes (pexophagy) (58–61), and iron (ferritinophagy) (62, 63). But recently, there has been a greater appreciation for the role of selective

autophagy and a plethora of other proteins and organelles have been described as selective autophagy targets including, lipid droplets (lipophagy) (64, 65), aggregates (aggrephagy) (66–69), and the nucleus (nucleophagy) (70–72), among others.

Selective autophagy relies on proteins called selective autophagy receptors to recognize the cargo and direct it to the autophagic machinery. Most selective autophagy receptors have canonical LC3-interacting regions that bind to the GABARAP/LC3 family of proteins anchored in the phagophore membrane, which serves as a recognition target (73, 74). There are a variety of different adaptor proteins that recognize specific proteins and cargo and deliver the cytoplasmic material to forming autophagosomes via their LC3-interacting region motifs. One of the most well characterized is sequestosome 1 (SQSTM1)/p62 (68, 69), a multifunctional scaffold protein that is involved in numerous processes including autophagy but also apoptosis (75, 76). Other cargo adaptor proteins include Nuclear Dot Protein 52 (NDP52), Optineurin (OPTN) and Tax1 Binding Protein 1 (TAX1BP1) (77) which are involved in mitophagy, nuclear receptor coactivator 4 (NCOA4) involved in ferritinophagy, and Neighbor of BRCA1 gene (NBR1) involved in several types of selective autophagy, all of which are finally degraded along with the cargo (50, 63, 78, 79). These selective autophagy mechanisms have been reviewed in detail elsewhere (2, 50, 77).

Noncanonical Autophagy

In addition to the canonical autophagy processes described above, it is now well recognized that autophagosomes can also be formed in the absence of some key ATG proteins. These alternative pathways are collectively termed "noncanonical autophagy" (2). For instance, various proapoptotic treatment or compounds have been shown to induce noncanonical BECN1-independent autophagy likely as an attempt to cope with stress (80–83). In addition, autophagic degradation that does not require other core autophagy proteins such as ULK1, ATG5, or ATG7 have also been reported (81, 82).

In addition to these alternative pathways, many ATG proteins also have autophagy-independent roles (84, 85). For instance, several ATG proteins are implicated in membrane-related functions (eg, endocytosis and phagocytosis), but also in modulation of host infection by pathogens, as well as inflammation and immune signaling, cell death, genomic stability, and cell proliferation (84, 85). LC3 proteins can also conjugate directly with phagosome membranes independent of the AMPK–mTOR–ULK signaling pathway in a process called LC3-associated phagocytosis. LC3-associated phagocytosis instead relies on ATG7 and RUBICON to facilitate macrophage-mediated clearance of apoptotic cells and modulates immunity through antigen presentation and pathogen clearance (86). The LC3 conjugation machinery is also involved in the loading and secretion of extracellular vesicles containing small noncoding RNAs which could be important in cell–cell communication (87). With numerous examples of ATG-independent autophagy combined with a growing number of ATG-regulated autophagy-independent processes, the definitions of canonical and noncanonical autophagy have become increasing complex. These realities make it difficult to implicate the process of autophagy, in its entirety, as either necessary or sufficient for different physiological/pathological states (84, 85). Rigorous studies must include manipulation of multiple regulators of the process while also carefully ruling out autophagy-like processes including LC3-associated phagocytosis.

Autophagy Acts as a Double-Edged Sword Across Tumor Types

Autophagy was first suggested to play a tumor suppressive role in cancer by Beth Levine's group which described Beclin 1 as a candidate tumor suppressor that is lost in human tumors (4). Additional preclinical studies have elucidated the mechanisms behind Beclin 1–mediated tumor suppression including its interaction with Bcl-2, an antiapoptotic protein, and regulation of Human Epidermal Growth Factor Receptor 2 (HER2) (discussed in detail later) (88, 89). However, contrary to this work, studies using The Cancer Genome Atlas human cancer sequencing data determined that Beclin 1 is encoded near the bona fide tumor suppressor, BRCA1 (90). Breast and ovarian tumors often present with concomitant *BECN1* (encodes the Beclin 1 protein) and *BRCA1* deletions, but rarely BECN1 deletions alone indicating BRCA1 is the primary tumor suppressor. Mutations in Beclin 1 complex proteins such as UVRAG, BIF1, and AMBRA have also been implicated in cancer, however these proteins all have autophagy-independent roles begging the possibility that the Beclin 1 complex might be tumor suppressive independent of its autophagy related functions (34, 91, 92).

Beyond Beclin 1, other studies that have shown that autophagy regulates normal cell homeostasis and prevents tumor progression. Healthy cells detect DNA damage and trigger cell death in the event of telomeric damage signals in order to maintain genomic stability and prevent uncontrolled cell growth (93). Cells that can escape programmed cell death often go on to trigger tumorigenesis. In normal human fibroblasts and epithelial cells, telomeric damage induces autophagy through the cGAS-STING pathway which can detect cytosolic DNA (94). Inhibition of autophagy in these models allowed cells to grow with genetic aberrations and unstable genomes. Autophagy-deficient cancer cells also experience cytokinesis failure resulting in cells with multiple nuclei and abnormal numbers of chromosomes (95). These studies indicate that autophagy is critical in check-point mechanisms that prevent the accumulation of genetic damage that can result in downstream tumorigenesis.

Several other studies have highlighted the dual role for autophagy in cancer, where it is tumor suppressive at early stages of tumor development but tumor promotional in established tumors (1–3) (Fig. 2). In mouse models of pancreatic ductal adenocarcinoma (PDAC), the loss of ATG5 increases the formation of premalignant PanINs (pancreatic intraepithelial neoplasia) but inhibits the progression of these PanINs to invasive and malignant PDAC (96). However, in established tumors, autophagy helps maintain glucose metabolism that supports the growth and progression of the tumor (97, 98). A similar dual role for autophagy has been seen in lung cancer models where the loss of *Atg5* or *Atg7* increases tumorigenesis but inhibits the progression of benign tumors to advanced disease (99, 100). Importantly, a shared limitation among these studies investigating autophagy inhibition on cancer growth is that most have only deleted 1 core autophagy protein (eg, ATG7, or ATG5, or BECLIN), and the effects observed may therefore be attributable to the autophagy-independent

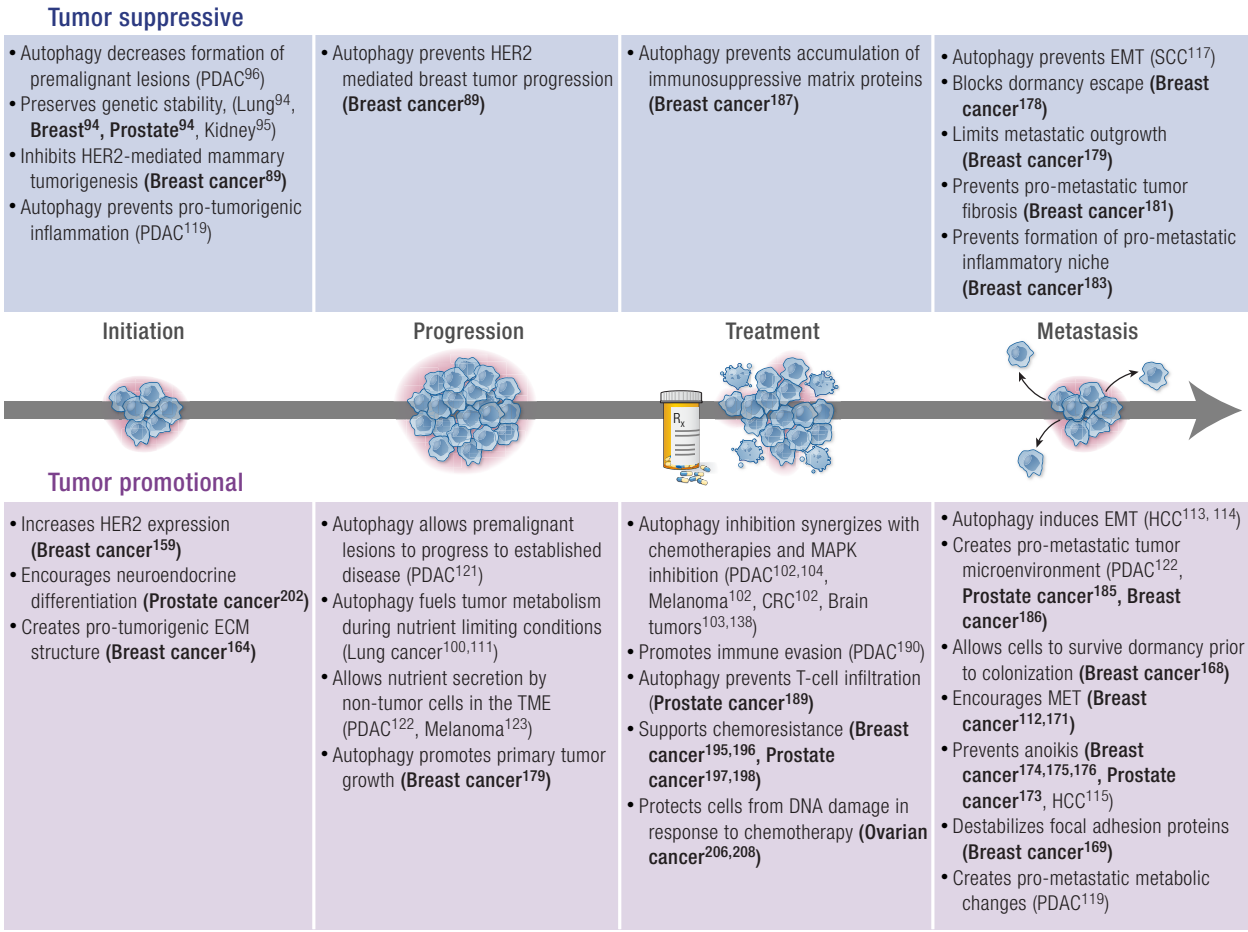

Figure 2. The tumor promotional and tumor suppressive effects of autophagy at different stages of cancer. Bold text highlights studies focused on endocrine-dependent tumors. Autophagy can play a cytoprotective role at the early stages of tumor development by maintaining genetic stability and therefore limiting tumorigenesis. However, it has also been shown to increase the surface expression of progrowth receptors, increase differentiation and alter the TME to increase tumorigenesis. Most studies indicate that autophagy promotes the progression of tumors through cancer intrinsic and extrinsic mechanisms including fueling tumor metabolism. In established tumors upregulated autophagy can render cells resistant to therapy by minimizing genetic damage and clearing damage inducing molecules like ROS. Interestingly, the effects of autophagy on metastasis have almost exclusively been studied in endocrine-dependent cancers and many suggest that autophagy promotes metastatic disease. Several studies in breast and prostate cancer models show that autophagy creates a prometastatic niche, increases EMT, and prevents anoikis. Abbreviations: PDAC, pancreatic ductal adenocarcinoma; ECM, extracellular matrix; CRC, colorectal cancer; SCC, squamous cell carcinoma; EMT, epithelial–mesenchymal transition; HCC, hepatocellular carcinoma.

noncanonical function of each gene (see "Noncanonical Autophagy").

In the following sections we first highlight the role of autophagy in developing, fueling, and exacerbating nonendocrine-dependent tumors and how these effects can be harnessed therapeutically. The tumor promotional role of autophagy in advanced tumors has led to several preclinical and clinical studies depicting that autophagy inhibition in combination with chemotherapy can cause tumor regression in melanoma, PDAC, lung, and brain tumors (96, 101–104).

We will then discuss the complex roles of autophagy in endocrine-dependent tumors and provide perspective for future directions in the field.

Autophagy and Tumor Metabolism

Autophagy acts as a quality control mechanism to break down damaged substrates such as misfolded proteins and damaged organelles and in turn generate metabolites that can be used for downstream processes (43, 105). These metabolites can fuel cells during nutrient deprivation and support their growth. Accordingly, autophagy is crucial for the survival of nontumor bearing mice under fasting conditions (100). Mice with inducible whole-body knockout of *Atg7* in adult animals cannot survive starvation for 24 hours due to hypoglycemia (100). These studies showed that liver glycogen stores are more depleted in $Atg7^{-/-}$ mice, indicating that alternative sources of nutrients are crucial to fueling the organism during starvation even in healthy adult mice.

The tumor microenvironment (TME) can be extremely nutrient limiting due to the lack of proper vascularization and desmoplasia and can make autophagy's role even more essential (106–109). In Kras-driven p53-deficient nonsmall cell lung cancer mouse models, the lack of autophagy does not affect the ability of tumors to develop but it is crucial for growth after the tumor is established. The autophagy pathway helps sustain metabolites like amino acids, pentose phosphate pathway intermediates, and alpha ketoglutarate (110). Glutamine and glutamate appear to be the most essential products since their exogenous supplementation allows Kras-driven autophagy-deficient lung tumors to continue to grow. This indicates that established tumors are autophagy-dependent and could be an ideal target for therapeutics.

Autophagy and metastasis

Aggressive cancer types eventually spread to secondary sites through the process of metastasis, which often results in increased morbidity. The process of cancer metastasis involves escaping from the primary tumor, entering circulation, invading a secondary site, and then colonizing this new site (111). During this multistep process, cells must overcome several barriers including nutrient stress, isolation, lack of substrate attachment, foreign microenvironments, immune surveillance, and cell death pathways to survive (111). Cancer cells can exploit several mechanisms including metabolic reprogramming, immunosuppression, and mechanical remodeling to support their journey from the primary to the secondary site.

Autophagy has been implicated in cancer metastasis in several different tumor types (Fig. 2). The process of epithelial to mesenchymal transition (EMT) allows cancer cells to develop invasive and migratory properties allowing primary tumor cells to escape and develop metastatic tumors at secondary sites (112). EMT is induced by autophagy in a transforming growth factor β–dependent manner in hepatocellular carcinoma models (113, 114). The cytoprotective effect of autophagy induction has been linked to anoikis resistance and increased lung colonization of metastatic hepatocellular carcinoma cells (115). An analysis of solid tumor samples across several tumor types also indicates that high LC3B expression is associated with increased tumor proliferation and invasiveness indicating that autophagy promotes tumor metastasis (116).

However, other studies have shown that decreased autophagy enhances tumor progression and migration indicating that autophagy has metastasis-preventing functions as well (117, 118). These studies showed that the accumulation of p62, an autophagy substrate, stabilized the EMT-regulating transcription factor, Twist1 and increased tumor migration in squamous cell carcinoma models. Interestingly, the extent of autophagy gene ablation seems to impact tumorigenic effects (119). In mouse models of PDAC, complete knockout of *Atg5* blocks tumorigenesis while monoallelic loss of *Atg5* promotes tumorigenesis and metastasis. These results indicate that the timing and extent of autophagy inhibition could be crucial to antitumor efficacy.

The tumor suppressive nature of autophagy in early stages of cancer and tumor promotional nature in established disease has been well documented in nonendocrine tumors but relatively understudied in endocrine-dependent tumors. On the contrary, the effects of autophagy on metastasis have been studied more extensively in endocrine tumors; these studies have been highlighted in depth in future sections.

Autophagy and the tumor microenvironment

Tumors are typically nutrient deplete due to the dense TME, hyperactive metabolism, and underdeveloped vasculature. To help survive these harsh conditions, tumor cells can utilize the benefits of autophagy in nontumor cells in the microenvironment via noncell autonomous mechanisms (120–123). In a *Drosophila melanogaster* model of malignant tumors, noncell autonomous autophagy supports tumor growth and invasion. This study revealed that blocking autophagy in the primary tumor reduces tumor burden but not invasiveness. However blocking autophagy in surrounding nontumor cells results in a more robust reduction in tumor growth and invasiveness (120).

The TME in pancreatic ductal adenocarcinoma (PDAC) is highly desmoplastic and contains an abundance of extracellular matrix proteins and pancreatic stellate cells that can interact with tumor cells (124). This dense TME contributes to highly nutrient limiting and hypoxic conditions which generally correlate with poor prognosis (106–108, 125). Work by Endo et al showed that pancreatic cancer stellate cells use autophagy to secrete extracellular matrix proteins and cytokines which influence the TME (122). Accordingly, coculturing PDAC cell lines with pancreatic cancer stellate cells increases the invasiveness of the PDAC cells. This study showed that treating the cocultures with the lysosome-mediated autophagy inhibitor, chloroquine (CQ), reduced growth of the PDAC cells, but had no effect on PDAC cells in monoculture. Importantly, the pancreatic TME is highly specific to this tumor type, but nevertheless these studies highlight the importance of autophagy levels in the TME on the growth of cancer cells, which could have major implications for other cancer types as well.

The tumor promotional effects of pancreatic stellate cells have also been linked to metabolite secretion (121). Pancreatic stellate cells secrete alanine in an autophagy-dependent manner which in turn fuels PDAC cell growth. Alanine is particularly important because it fuels the TCA cycle and downstream synthesis of lipids and nonessential amino acids. Mice with whole body knockout of *Atg7* also have lower levels of circulating arginine, a nonessential amino acid involved in mTOR activation and protein synthesis (123). Poillet-Perez et al (123) showed that mouse melanoma cells implanted into autophagy deficient C57Bl/6J mice resulted in smaller tumors than their autophagy-competent counterparts. Tumor growth was rescued with exogenous supplementation of arginine, indicating that host autophagy provides key tumor supporting metabolites.

Targeting autophagy pharmacologically

Together these studies highlight the tumor promotional role of autophagy in established tumors and have resulted in the launch of many preclinical and clinical trials with autophagy inhibitors (6, 126–129). Current clinical trials primarily utilize CQ and hydroxychloroquine (HCQ) which are approved for use in humans as antimalarials and to treat rheumatoid arthritis (130–132). They inhibit autophagic flux by targeting autophagosomal fusion with lysosomes as well as lysosomal function itself. Recently, the specific lysosomal target of HCQ and its derivatives was identified as palmityl-protein thioesterase 1 (133). One of the first uses of CQ to treat cancer was in small study of 18 glioblastoma patients where the patients treated with CQ in addition to standard of care survived significantly longer than the control group of patients (134). Strikingly, 4/9 CQ-treated patients survived up to the 2-year follow-up timepoint while all 9 patients in the control group had succumbed to the disease. In another study, HCQ was combined with erlotinib, an EGFR tyrosine kinase inhibitor, to bolster antitumor response in patients with nonsmall cell lung (135). While both studies used CQ and HCQ for their antineoplastic properties, they did show that these drugs are safe and well tolerated. Since then, other studies have used CQ and HCQ specifically for autophagy inhibition to boost antitumor response in advanced solid tumors, melanoma and PDAC (129, 136). In a phase I dose escalation study 27 advanced solid malignancy and 12 metastatic melanoma

patients were treated with HCQ combined with the mTOR inhibitor, temsirolimus. In this study, a majority of the patients achieved stable disease, however no partial responses were observed (129). The efficacy of autophagy inhibition has been more promising in PDAC patients (128, 136). In a phase I/II trial of 31 preoperative patients treated with gemcitabine (2 doses) and HCQ (continuous 31 day treatment), 61% of patients showed a decrease in the prognostic biomarker CA 19-9. Patients with decreased CA 19-9 had better overall and disease-free survival (136). A long-term analysis of patients in this study showed that 31% of patients survived past 5 years post-diagnosis which is better than the 11% 5-year survival rate for most PDAC patients (128, 137). All of these studies showed that the combination of autophagy inhibition with chemotherapy agents are well tolerated and safe.

Combining autophagy inhibition with MAPK pathway inhibition has been particularly promising. It was shown that MEK/ERK inhibition resulted in increased autophagy flux *in vitro* (102, 104), and that combining ERK inhibitors with CQ resulted in synergistic inhibition of cell growth and a reduction in patient derived xenografts tumor growth (102, 104) as well as in a case study with 1 patient with BRAFi resistance (104). Similarly, cell lines generated from BRAF mutant central nervous patient tumors are highly autophagy dependent and show robust sensitivity to CQ in culture (138). The authors go on to show that treating a single patient with BRAF (V600E) mutant brainstem ganglioglioma with CQ and the BRAF (V600E) inhibitor, vemurafenib, improved her clinical response and stopped tumor growth. Another study by the same group showed that in pediatric brain tumors, autophagy inhibition was able to circumvent resistance to vemurafenib in vitro, a strategy that seems successful in a clinical case study of a single patient reported in the same paper (103). Recently, a preclinical study in pancreatic cancer reported that elevated autophagy in response to treatment with the MEK inhibitor trametinib was required for ferritinophagy, a process which provides iron to the cancer cells, thereby enhancing mitochondrial respiration even under stressed conditions (139). This could explain, at least partly, the synergistic effects observed when MEK/ERK inhibitors are combined with autophagy blockade.

A common limitation of all the clinical studies mentioned is the extremely small number of patients. Moreover, it is unclear how well autophagy was blocked in each patient as the field still lacks reliable biomarkers to determine autophagy inhibition in tumors. Nevertheless, these findings and others led to the conduction of clinical trials combining MEK/ERK inhibitors with autophagy inhibition (NCT03825289, NCT04214418, NCT03979651, NCT03754179, NCT04566133, NCT022 57424). Most recently, published results from the BAMM trial in melanoma patients combining BRAF inhibition (dabrafenib) with MEK inhibition (trametinib) and HCQ showed that this treatment combination was safe and, with a nearly 86% response rate, might be of benefit for patients with increased serum lactate dehydrogenase (LDH) levels and prior treatment (NCT02257424). Interestingly, the study did not report any survival benefit with the addition of autophagy inhibition as the median progression free survival was reported as 11.2 months compared with 11.1 months in prior studies with just dabrafenib and trametinib (140). These results strongly suggest that despite high response rates, acquired resistance to autophagy inhibition may be a significant hurdle for this therapeutic strategy. The authors also highlight that the demographics of the BAMM trial included more patients with high serum LDH levels and therefore worse prognosis than prior trials with dabrafenib/trametinib alone. Moreover, this trial was nonrandomized and had an early closure partly due to lack of accrual (101).

It is still unclear how well CQ and HCQ successfully and specifically inhibit autophagy. Recent studies have developed more potent and targeted autophagy inhibitors such as ULK1 inhibitors, Vps34 inhibitors, and more specific palmitoyl-protein thioesterase 1 inhibitors including DC661 and GNS561 that could be useful in studying the specific effects of autophagy in preclinical models and provide improved efficacy in clinical trials (133, 141).

Together these studies highlight the safety and potential efficacy of targeting autophagy in a broad panel of tumor types. However, larger cohorts of patients are needed to draw definitive conclusions about the future of this targeted therapeutic strategy. Moreover, some studies suggest select patient populations may benefit, namely, patients with high serum LDH levels in the BAMM trial; a hypothesis that can only be tested in larger trials. And finally, the field is in desperate need of effective biomarkers to determine how much autophagy is inhibited at safe doses of HCQ, but also with safe doses of new autophagy-targeting agents currently moving through the pipeline.

Autophagy in Endocrine-dependent Tumors

Endocrine tissues play an important role in the regulation of biological functions, including heart rate, reproduction, stress response, and metabolism, by secreting hormones that elicit responses in specific cell types (142). Cancers that respond to hormones, including breast, prostate, endometrial, thyroid, and ovarian cancer, are classified as endocrine-dependent tumors (143). Sex hormones have been shown to stimulate the growth of human mammary and prostate cancer cells in particular via their interaction with their receptor (eg, estrogen receptor and androgen receptor) (144–146). Their binding modulates gene expression either via direct or indirect genomic signaling, resulting in downregulation of death signaling pathways while upregulating growth pathways (147). In addition, sex hormones also have nongenomic signaling, and can for instance interact with Ras and activate the MAPK pathway (144). Hormones and hormone receptors play a particularly crucial role in breast cancer where nearly 75% of diagnosed cases are hormone receptor-positive indicating that they express hormone-sensitive estrogen receptors (ER +) or progesterone receptors (PR+) (148). Therapeutic strategies for these tumors include blocking hormone–receptor interactions using tamoxifen or reducing circulating hormone levels using aromatase inhibitors (149). In prostate cancer, blocking androgen signaling—and subsequent transcriptional activity—represents the standard first-line treatment for advanced disease (150). As early as 1941, the work of Huggins and Hodges—based on the observation that testosterone regulates the growth of prostate glands—started treating prostate cancer patients with either surgical castration or medical castration (via estrogens) (151). Treatment with less undesirable side effects and targeting gonadotropin-releasing hormone were later developed. Gonadotropin-releasing hormone agonists (eg, leuprolide, bruserelin, and goserelin) and antagonists (eg, cetrorelix, ganirelix, and teverelix) block the release of luteinizing hormone and follicular stimulating hormone involved in the production and release of testosterone in the testes, thereby resulting in a drastic decrease in testosterone

levels (152). Hormone modulation in the form of androgen deprivation therapy also plays a critical role in treating metastatic prostate cancer (150, 153). Altered hormone signaling can also result in more aggressive forms of disease or increase the risk of developing disease. Prostate cancer can develop into castration-resistant prostate cancer that is no longer responsive to the reduction of testosterone levels. Exogenous hormones in the forms of oral contraceptives and hormone replacement therapy can also influence the risk of developing breast and endometrial cancers (154–156). Autophagy has been implicated in several stages of endocrine-dependent tumors including initiation, progression, metastasis, and response to treatment but its role in these processes remains unresolved (Fig. 2).

Initiation and Progression of Endocrine-Dependent Tumors

Tumor intrinsic effects of autophagy

Like other tumor types, autophagy has been implicated in the initiation and progression of endocrine-dependent tumors via tumor intrinsic mechanisms. A number of breast cancer cell lines are sensitive to loss of core autophagy genes and sensitive to autophagy targeting drugs (157, 158). Autophagy has been implicated as a positive regulator of HER2+ breast cancer. A recent study showed a nearly complete block in HER2+ breast cancer tumorigenesis upon knock out of FIP200 in the MMTV-Neu breast cancer mouse model (159). These results were also replicated in mice with the FIP200 mutant knock in mutation that prevents its binding to ATG13 and therefore its ability to regulate autophagy, but still preserves the autophagy-independent functions of FIP200 (160). This indicates that reduced tumorigenesis is due to the autophagy-specific function of FIP200 rather than its autophagy-independent roles. The overexpression of HER2 promotes breast tumorigenesis and progression (161, 162). Specifically, plasma membrane expression of HER2 in breast cancer is associated with more aggressive phenotypes and worse prognosis (163). Autophagy facilitates HER2 localization on the plasma membrane in breast cancer cells (159) and blocking autophagy, via regulation of FIP200, in these models causes HER2 to be released out of the cell through extracellular vesicles instead of trafficked to the plasma membrane for surface expression. Loss of autophagy causes HER2 to traffic from the Golgi to endosomes instead of the plasma membrane resulting in endosomal-mediated HER2 release into the extracellular space through small extracellular vesicles. Interestingly, this work also showed that blocking the release of the extracellular vesicles using siRNAs for Rab27a cannot rescue HER2 expression at the cell surface indicating that simply accumulating HER2 in the cell is not sufficient to direct its localization to the plasma membrane. Moreover, a strength of this study is that it includes the manipulation of other core autophagy genes including Atg5 in their mouse models and ATG13 in human cell lines, which recapitulate the findings with FIP200 knockout. This study provides an elegant model by which autophagy facilitates mammary tumorigenesis via regulation of HER2.

While these studies implicate autophagy in breast tumorigenesis, other studies have defined a tumor suppressive role for the recycling process. For example, Beth Levine's group found that HER2 interacts directly with the upstream autophagy regulator, Beclin 1, resulting in decreased autophagy (89). Moreover, these studies showed that increasing basal autophagy via a knock in mutation in Beclin 1 inhibits mammary tumorigenesis both in vitro and in vivo xenograft mouse models (89). This study shows that autophagy promotes early stages of tumorigenesis in breast cancer similar to the observations in lung and pancreatic cancer (96, 99). However, a weakness of this study is that they only manipulate autophagy using Beclin1 and do not validate their findings with other autophagy genes. Interestingly, these studies in breast cancer did not observe the dual role of autophagy as has been seen in other tumor types. Nonetheless, these studies, with seemingly opposite results regarding the role of autophagy and HER2 +-positive breast cancer described in the above study, highlight the need for additional mechanistic insight into the roles of autophagy in this endocrine-dependent tumor type.

Tumor extrinsic effects of autophagy

Autophagy has also been implicated in tumor cell extrinsic mechanisms that regulate tumor progression in endocrine-dependent tumors (164, 165). The TME typically consists of fibroblasts, extracellular matrix components, immune cells, and abnormal vasculature and can play an important role in nutrient availability and immunogenicity. Stromal fibroblasts secrete collagen and contribute to the stiffness of the TME which in turn influences immune cell infiltration as well as cytokine production and desmoplastic tumors generally correlate with poorer prognosis (166, 167). Autophagy in nontumor cells within the TME can influence tumor initiation and progression by affecting the TME desmoplasia in nonendocrine dependent tumors as mentioned earlier, but these effects are also seen in endocrine-dependent tumors (164, 165). For example, autophagy in the stromal fibroblasts of the mammary TME promotes mammary tumor growth (164). Rudnick et al (164) showed that tumors implanted with *Atg12*-deficient mammary fibroblasts were significantly smaller than tumors implanted with autophagy-competent fibroblasts. These effects were linked to altered collagen architecture and an overall reduced level of collagen deposition in mouse mammary fibroblasts that lack autophagy compared with the wild-type fibroblasts. A similar tumor promotional effect of autophagy in the TME has also been shown in triple negative breast cancer (165). Cancer-associated fibroblasts isolated from patients with triple negative breast cancer exhibit higher levels of autophagy pathway associated proteins, LC3-II and Beclin 1, compared with matched normal fibroblasts harvested at tumor adjacent sites. These studies indicate that autophagy in tumor resident fibroblasts can influence the TME and the outgrowth of adjacent tumor cells in breast cancer. Systemic administration of autophagy inhibitors would likely target tumor intrinsic and extrinsic autophagy simultaneously which could improve antitumor effects and patient outcomes.

Metastasis of Endocrine-Dependent Tumors

Prometastatic functions of autophagy

In contrast to the ever-growing body of literature exploring the roles of autophagy in tumor initiation and primary tumor growth, there have been few studies to specifically investigate the role of autophagy in metastasis. Interestingly, most studies tackling these questions have focused on endocrine-dependent tumors such as breast and prostate cancer and have shown tumor promotional and tumor suppressive functions (Fig. 3).

Autophagy supports metastasis at several stages in the process including the acquisition of motile phenotypes and avoiding detachment-induced cell death, otherwise known as anoikis (112, 168, 169). Tumor cells that can remain dormant

at secondary sites can often result in metastatic disease recurrence, a process that can be modeled in vitro where exposure to basement membrane extract and collagen induces dormant breast cancer cells to re-enter a proliferative state (168). Using this in vitro dormancy model, Vera-Ramirez et al (168) found an increase in autophagy when the cells remained in a solitary dormant state compared with a proliferative state. Moreover, inhibiting autophagy with the lysosomal autophagy inhibitor, HCQ, reduced the viability of dormant cells and decreased lung metastatic burden in vivo by preventing dormant cells from acquiring a more proliferative phenotype. Interestingly, these effects were only recapitulated with genetic manipulation of ATG7 but not BECN1 and the effects of autophagy inhibition on lung metastasis were dependent on the fibrotic conditions of the lung, suggesting that these effects are context specific. The cytoprotective activity of autophagy in dormant cancer cells could be due to the activity of spleen tyrosine kinase (112). Metastasis often requires cells to acquire a migratory mesenchymal-like phenotype through EMT and then revert to an epithelial phenotype through mesenchymal to epithelial transition to colonize a secondary site (170). Work by the Geahlen group showed that RNA processing complexes called P-bodies tend to accumulate in the cytoplasm during EMT (171). In a follow-up study by the same group, it was found that P-bodies are cleared during mesenchymal to epithelial transition by spleen tyrosine kinase in an autophagy-dependent manner (112). Autophagy inhibition via ATG7 knockout leads to an accumulation of P-bodies and a stabilized mesenchymal phenotype therefore preventing cells from metastasizing. The authors showed that *Atg7* deletion had minimal effects on primary 4T1 mammary tumors but drastically blocked metastasis, indicating that autophagy plays a critical role in allowing dormant mesenchymal-like cancer cells to revert to an epithelial state to induce metastasis. Especially notable is that these findings were replicated by inhibiting autophagy pharmacologically with R406 and fostamatinib (spleen tyrosine kinase inhibitors known to inhibit autophagy) in vivo. Autophagy also promotes tumor cell migration and subsequent metastasis by promoting focal adhesion disassembly (169). Knocking down *Atg5* in 4T1 mammary tumor cells causes dysfunctional focal adhesion assembly, a process crucial to cell migration. An important strength of this study is that the results were replicated in *Atg7*-deficient mice, indicating that the prometastatic effects observed were likely autophagy dependent. The authors showed that autophagic degradation of paxillin, a focal adhesion protein, is required for cell migration and invasion indicating that autophagy plays a prometastatic role.

Cells that lack , extracellular matrix attachment typically undergo a specialized form of cell death called anoikis

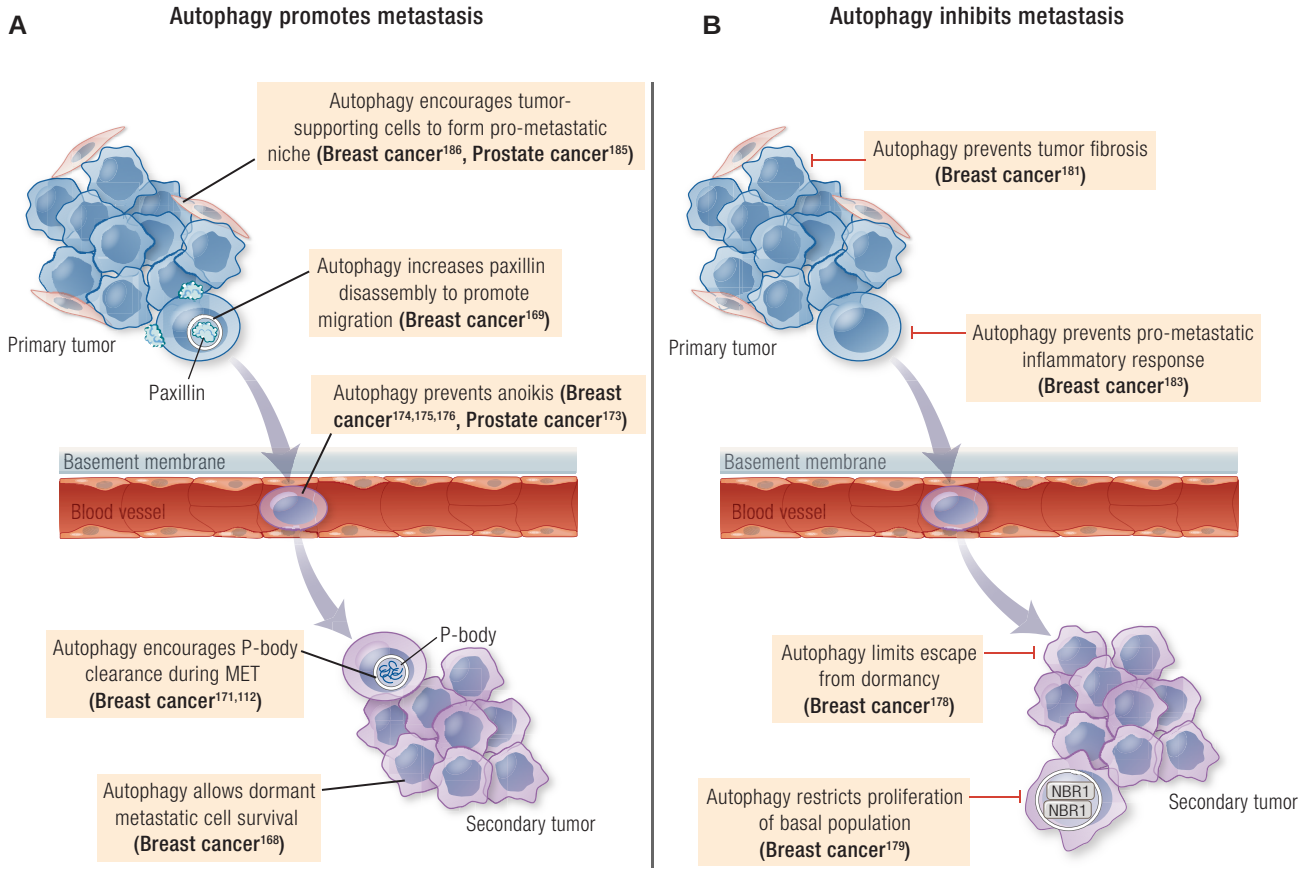

Figure 3. Autophagy has opposing roles in metastasis of endocrine-dependent tumors. (Created with BioRender.Com). (A) Autophagy promotes metastasis. The process of metastasis involves detachment from the primary tumor, acquiring migratory phenotypes, entering circulation, and then colonizing a secondary site. Autophagy can degrade paxillin, a focal adhesion protein, which can enable breast cancer cells to become more migratory. Once in circulation, it can prevent anoikis, which is a type of apoptosis that is activated in response to matrix detachment. Cells can then reacquire endothelial phenotypes and remain dormant at secondary sites using cytoprotective autophagy. (B) Autophagy inhibits metastasis. Autophagy has been shown to decrease tumor fibrosis and inflammation that can contribute to a prometastatic TME. It has also been shown to prevent metastatic cells from being able to escape dormancy and expand at the secondary site.

(172). Autophagy can support metastasis by protecting cells from anoikis and promote cell survival (173). Mammary epithelial cells that are unable to attach to a surface have upregulated autophagy as measured by LC3 puncta abundance (174). Reducing ATG5 and ATG7 expression in these detached cells using siRNAs increases apoptosis. The induction of cytoprotective autophagy in response to cell detachment has been linked to Protein Kinase RNA-Like ER Kinase (PERK) and I κB kinase complex (IKK) (175, 176). PERK induces autophagy and an antioxidant stress response which can both protect cells from detachment-induced cell death (175). In 3D models of mammary epithelial cells, IKK inhibition reduces autophagy and increases anoikis-induced cell death (176). Prostate cancer cells that are anoikis-resistant also exhibit upregulated autophagic flux compared with their parental counterparts (173). Yu et al identified a mechanism by which cell migration–inducing protein (CEMIP) induces protective autophagy in anoikis-resistant prostate cancer cells. They showed that knock down of CEMIP decreased autophagy as well as pulmonary metastasis in vivo. However, blockade of more specific autophagy-related genes (eg, *Atg7* or *Atg5*) was not performed. These studies indicate that autophagy plays a cytoprotective role and prevents attachment-free cells from undergoing anoikis.

Antimetastatic functions of autophagy

While there is a large body of literature including fundamental studies, preclinical, and even clinical, work indicating autophagy promotes the survival and growth of the primary tumor and as described above tumor dormancy, in recent years there has been a growing body of seemingly paradoxical literature suggesting that autophagy inhibits the metastatic potential of breast cancer cells (6, 100, 101, 168, 177, 178, 179–181). Elegant work by Dr. Debnath and colleagues revealed that even though loss of autophagy via ATG12 or ATG5 knockout impeded the growth of primary mammary tumors in a MMTV-PyMT model, the loss of autophagy also resulted in larger metastatic lesions (179). Interestingly, the number of metastatic lesions were comparable to the wild type controls. The prometastatic effect of autophagy inhibition is due to the accumulation of the autophagy substrate NBR1 and independent of p62 accumulation. A strength of this study is that both ATG5 and ATG12 were manipulated to confirm that the observed tumor growth defects and antimetastatic effects were indeed autophagy inhibition-related. Other studies have also shown that loss of the upstream autophagy-regulating kinase ULK1 promotes cell migration and invasion of MDA-MB-231 breast cancer cells (181). The lack of ULK1 function causes more fibronectin deposition in xenograft models of breast cancer, indicating that autophagy can prevent tumor fibrosis which is known to encourage metastasis (182). Accordingly, knockdown of ULK1 increases pulmonary metastases in this model. Another study showed that breast cancer cells in hypoxic conditions that downregulate ULK1 have attenuated mitophagy, the selective autophagic degradation of mitochondria, which results in increased bone metastasis of breast cancer cells (183). The lack of ULK1-mediated mitophagy results in the accumulation of dysfunctional mitochondria and reactive oxygen species (ROS), which activate the inflammasome and subsequent bone metastasis. However, these studies only block autophagy by ULK1 deletion and no other autophagy pathway genes. In addition, the studies did not include an evaluation of ULK2 manipulation which has been shown to compensate for ULK1 loss (184). Negative regulation of ATG5-mediated macroautophagy by chaperone-mediated autophagy has also been shown to promote breast cancer metastasis (180). 6-Phosphofructo-2-kinase/fructose 2, 6-biphosphatase 3 (Pfkfb3) is an enzyme involved in glycolysis, cell division, and apoptosis regulation. In breast cancer models, Pfkfb3 expression is inversely correlated with autophagy as measured by LC3 puncta and LC3-tandem reporter flux (178). In this study, knocking down ATG3 or ATG7 in breast cancer stem cells increases Pfkfb3 expression, cell proliferation, and allows cells to escape metastatic dormancy. This indicates that autophagy suppresses aberrant Pfkfb3 expression and limits the ability of breast cancer cells to escape dormancy and regain proliferative capacity to establish metastatic disease.

Autophagy in the microenvironment influences metastasis

Autophagy in fibroblasts and endothelial cells in the TME can also support metastasis of endocrine-dependent tumors (185, 186). In a coculture model of castration-resistant prostate cancer cells with endothelial cells, it was found that the tumor supporting endothelial cells induce autophagy in neighboring cancers cells, which causes downregulation of androgen receptor expression, destabilization of paxillin, and increased invasive properties of the cancer cells (185). In breast cancer models of TME and cancer cell interactions, fibroblasts used autophagy to convert into activated cancer-associated fibroblasts that are tumor promotional. Once the cancer-associated fibroblasts are in their activated state they induce EMT in cancer cells to promote migration and invasion (186). These studies suggest that autophagy can also have tumor-extrinsic roles in promoting metastasis.

Treatment of Endocrine-Dependent Tumors

The role of autophagy in endocrine tumor response to treatments is also controversial. Different studies have shown autophagy can both increase treatment-induced tumor killing or impede it.

Autophagy and endocrine tumor response to immunotherapy

Autophagy is important for tumor to immune cell crosstalk and can impact the tumor response to immunotherapy treatments. Patients with triple negative breast cancer typically respond poorly to immunotherapy treatments including anti-PD1 and anti-PDL1 antibodies due to restricted cytotoxic T-cell activity (187). A cytotoxic T-lymphocyte assay using a large panel of autophagy genes showed that autophagy-deficient MDA-MB-231 cells experience significantly less cell death compared with their autophagy-competent counterparts. In an immunocompetent BALB/c mouse model, it was shown that allografted 4T1 mammary carcinoma cells lacking Atg5 or Beclin 1 grew faster and had lower infiltrating CD4+ and CD8+ T cells than their wild-type counterparts. Accordingly, anti-PD1 showed minimal effect in autophagy-deficient tumors. Inhibition of Tenascin C (TNC), an extracellular matrix protein and substrate of selective autophagy, increases tumor cell killing by cytotoxic T cells, indicating that TNC accumulation confers an immunosuppressive behavior of autophagy-deficient tumor cells. Blocking TNC in autophagy-deficient MDA-MB-231 cells successfully sensitize tumors to T-cell killing when treated with anti-PDL1 antibodies. Among the strengths of this study, the authors validate

their findings using several autophagy genes in their in vitro and in vivo models including Atg5, Atg7, and Beclin 1. TNC has also been implicated in immune surveillance in prostate cancer (188). Prostate cancer stem cells use TNC to inhibit T-cell proliferation. Together, these breast cancer and prostate cancer studies indicate that autophagic substrates can affect immunosurveillance.

Interestingly, autophagy inhibition has also been implicated in promoting immune infiltration and immunogenicity in prostate cancer. Multityrosine kinase inhibitors lead to increased cell killing in several androgen receptor positive and negative prostate cancer cell lines in vitro and in vivo (189). These studies noted that the phase I-cleared multityrosine kinase inhibitor ESK981 also blocks autophagy, which was accompanied by an antitumor immune response in an immune competent prostate cancer mouse model. In support of these findings, the proimmunogenic effects of autophagy inhibition have also been observed in PDAC, where loss of autophagy increases MHC-I expression by blocking its degradation in lysosomes (190). Consequently, autophagy inhibition improves antitumor T-cell activity resulting in PDAC regression. These proimmunogenic effects of autophagy inhibition in PDAC and prostate cancer contradict with the breast cancer studies, where MHC-I levels remained unchanged with autophagy inhibition. The alternative findings between these studies suggest context specific effects of autophagy on immune evasion.

Autophagy in acquired therapy resistance

Cancer cells can acquire resistance to targeted therapies, chemotherapies, or hormonal therapies by employing mechanisms such as drug efflux, apoptosis inactivation, angiogenesis, and increasing DNA repair mechanisms (191–194). Autophagy has also been implicated as a cytoprotective mechanism to escape therapy-induced cell death in endocrine-dependent cancer cells by maintaining metabolic homeostasis and minimizing cellular damage. A study in tamoxifen-resistant MCF7 breast cancer cell lines, showed that knocking down ATG7 or treatment with CQ restored drug sensitivity (195). This effect was linked to the upregulation of the metastasis-associated 1 protein, which contributed to increased autophagy flux. In further support of this study, work by Zhou et al showed that autophagy inhibition and chemotherapy synergize in xenograft models of breast cancer (196). They showed that liensinine, a novel inhibitor of autophagy and mitophagy, when combined with doxorubicin treatment caused apoptosis in breast cancer cells by triggering mitochondrial fission.

Similar autophagy-mediated chemoresistance effects have been observed in prostate cancer studies (197, 198). Prostate cancer is often associated with genetic modification of the tumor suppressor gene *PTEN*, which negatively regulates the PI3k–Akt pathway (199). While AKT pathway inhibitors such as AZD5363 have high efficacy in some prostate cancer cell lines others demonstrate resistance. In a study by Lamoureux et al, AZD5363 was found to activate autophagy in resistant prostate cancer cell lines (198). Cotreatment of AZD5363 with Bafilomycin A1, a lysosome acidification inhibitor, or CQ synergized to induce apoptosis of resistant cells both in vitro and in xenograft models of prostate cancer indicating that autophagy acts as a mechanism of chemoresistance. Attenuated autophagy was also found to increase apoptosis in castration-resistant prostate cancer cells that are resistant to the mTOR inhibitor, everolimus. This phenomenon was linked to upregulated Nitrogen permease regulator-like 2 (NPRL2), which when knocked down attenuated autophagy. While NPRL2 has previously been described as a tumor suppressor, this study proposes that NPRL2 supports tumor growth and chemoresistance by enhancing autophagy (200, 201).

Autophagy has also been implicated in the development of castration-resistant prostate cancer (202). In hormone refractory prostate cancer cells hypoxia induces neuroendocrine differentiation and reduces expression of repressor element-1 silencing transcription factor (REST). REST is a key regulator of androgen receptor-mediated gene expression and EMT in prostate cancer (203, 204). This process of neuroendocrine differentiation requires the activation of autophagy, a downstream target of REST. The lack of autophagy-regulated homeostasis can also cause cancer cells to undergo damage in the form of endoplasmic reticulum stress (205). Targeting autophagy in a genetically engineered mouse model of prostate cancer lacking PTEN exclusively in the prostate leads to slower tumor progression. Interestingly, ATG7 deletion causes an effect in both castration-naive and the more aggressive castration-resistant tumors, although the effects were more dramatic in the former. These studies attribute the observed growth defects to increased estrogen receptor stress and reduced androgen receptor signaling.

Autophagy is also induced in response to therapy in ovarian cancer models (206). Cells that have dysfunctional BRCA genes rely on alternative DNA repair mechanisms such as poly adenosine diphosphate ribose polymerase to mitigate DNA damage and continue to grow (207). This has led to the use of poly adenosine diphosphate ribose polymerase inhibitors in these cancer types to limit their ability to evade DNA damage-induced cell death. One study showed that 9 ovarian cancer cell lines treated with olaparib exhibited increased LC3 puncta and autophagic flux. Combination treatments of olaparib and CQ or LYS05, a CQ dimer, significantly decreased cell viability in ovarian cancer cell lines when compared with single treatment regimens. These synergistic effects were linked to exacerbated ROS and DNA damage accumulation in response to autophagy inhibition. The proapoptotic effect of excessive DNA damage in response to combination therapy has been linked to the upregulation of p21, which plays an important role in inducing cell cycle arrest, senescence, and preventing differentiation (208–211). In cisplatin-resistant ovarian cancer cells, treating cells with CQ and cisplatin together bolsters cell death. It was found that CQ increased DNA damage as measured by phosphorylation of histone H2AX. These 2 studies indicate that combining chemotherapies with autophagy inhibition could be a promising therapeutic approach in ovarian cancer.

Clinical trials in endocrine-related tumors

On ClinicalTrials.gov there are currently 115 trials using HCQ or CQ to target autophagy in all cancer types. A detailed summary of the clinical trials across tumor type has been reviewed elsewhere (6, 126). Of these, 17 include endocrine-dependent tumors (Table 1). These trials are underway based on several studies indicating the antitumor effects of autophagy inhibition, although, as noted earlier, there is still controversy regarding when autophagy may promote tumor growth or inhibit it. Initial promising results from these studies indicate a low incidence of serious adverse side effects

Table 1. Clinical trials of autophagy inhibition in endocrine-related tumors

Tumor type	Status	Autophagy inhibitor	Additional treatment	Clinical trial phase	Clinical response	Serious adverse events	Clinical trial ID
Ductal carcinoma in situ	Completed	CQ (2 doses)	n/a	I/II	Change in lesion diameter measured by MRI Low dose = 6% (SD 19.8) High dose = 43% (SD 39)	Low dose: 0/7 (0%) High dose: 0/5 (0%)	NCT01023477
Breast cancer stages I-IV	Recruiting	HCQ	Letrozole, Palbociclib	I/II	Ongoing	Ongoing	NCT03774472
Advanced breast cancer Solid tumor	Recruiting	HCQ (3 doses)	Abemaciclib, Faslodex, Anastrazole, Letrozole	I	Ongoing	Ongoing	NCT04316169
Breast cancer	Recruiting	HCQ	Abemaciclib	II	Ongoing	Ongoing	NCT04523857
Breast cancer	Recruiting	HCQ	Avelumab, palbociclib	II	Ongoing	Ongoing	NCT04841148
Breast cancer Stage IIB	Recruiting	HCQ	Everolimus	II	Ongoing	Ongoing	NCT03032406
Breast cancer ER+	Terminated	HCQ	n/a	I	Not analyzed (insufficient data)	Not analyzed (insufficient data)	NCT02414776
Breast cancer	Terminated	HCQ	Ixabepilone	I/II	Not analyzed (insufficient data)	1/6 (16.67%)	NCT00765765
Breast cancer	Unknown	HCQ	n/a	II	Not analyzed (insufficient data)	Not analyzed (insufficient data)	NCT01292408
Breast cancer Invasive breast cancer	Unknown	CQ	n/a	II	Data not available	Data not available	NCT02333890
Prostate cancer Melanoma Renal cell cancer Advanced malignant solid neoplasm	Active, not recruiting	HCQ	Akt inhibitor MK2206	I	Data not available	Data not available	NCT01480154
Prostate cancer recurrent Solid tumor, adult	Recruiting	HCQ	Metformin, sirolimus, nelfinavir, dasatinib	I/II	Ongoing	Ongoing	NCT05036226
Prostate cancer	Recruiting	HCQ	SUBA-itraconazole	I/II	Ongoing	Ongoing	NCT03513211
Prostate cancer recurrent	Recruiting	HCQ Sulfate	n/a	II	Ongoing	Ongoing	NCT04011410
Prostate cancer	Terminated	HCQ	Docetaxel	II	Not analyzed (insufficient data)	3/11 (27.27%)	NCT00786682
Prostate cancer	Terminated	HCQ	Abiraterone ABT-263	II	Not analyzed (insufficient data)	1/13 (7.69%)	NCT01828476
Prostate carcinoma	Terminated	HCQ	n/a	Early phase I	Data not available	Data not available	NCT02421575
Prostate cancer	Unknown	HCQ	n/a	II	Data not available	Data not available	NCT00726596

Abbreviations: CQ, chloroquine; HCQ, hydroxychloroquine; MRI, magnetic resonance imaging; SD, standard deviation; ER+, estrogen receptor positive; SUBA, super bioavailable.

(NCT01023477). Another study of 6 patients combining HCQ and ixabepilone, a microtubule inhibitor, also showed low incidence of serious adverse events (16.67%) (NCT00765765). Interestingly, in prostate cancer patients, the combination of docetaxel with HCQ caused some serious adverse events (27.2%). Although results do show that autophagy inhibitors are well tolerated overall, there have yet to be results indicating the efficacy and increased response rate. There are still 9 ongoing trials to target autophagy across endocrine-dependent tumors and most include HCQ in combination with other targeted agents. The results of these trials will be critical to determine if autophagy inhibition is a viable therapeutic strategy to treat endocrine-dependent tumors. Given the preclinical data suggesting it may have divergent roles in the primary tumor compared with metastatic lesions, more trials may be necessary to understand these juxtaposing roles of autophagy in patient tumors.

Perspectives

The autophagy field has exploded over the past few decades expanding our understanding of the fundamental mechanisms, upstream regulators, and the complex roles of autophagy in disease pathologies (Fig. 1). While there was once a controversy regarding the role of autophagy in cancer, the cumulative work of the field indicates that controversy may have a resolution at least in nonendocrine-dependent tumors. Multiple studies in pancreatic cancer and lung cancer support distinct roles for autophagy at different stages of tumorigenesis. In these tumor types, autophagy is tumor suppressive in early stages of tumorigenesis due to its key roles in maintaining quality control and genomic stability in normal cells and precancerous lesions. In established pancreatic and lung cancers autophagy promotes tumor cell metabolism and fuels cancer growth via tumor cell autonomous and nonautonomous mechanisms. These tumor promotional roles of autophagy have also been observed in other nonendocrine-dependent tumors including melanoma, hepatocellular carcinoma, and brain cancers. This point is highlighted in Fig. 2. Together these studies have led to the launch of many clinical trials. But these trials, even with a plethora of supporting preclinical studies, are still plagued by the lack of good biomarkers that can indicate the amount of autophagy inhibition in patient tumors, the lack of understanding about which patients have autophagy-dependent tumors, acquired mechanisms of resistance to autophagy inhibition, and the lack of specific and potent autophagy blocking therapeutics. Completed clinical trials show promising results regarding safety; however, these other hurdles will need to be addressed before autophagy inhibition shows any efficacy in patients to reduce tumor burden and extend lifespan.

While the controversy is nearly resolved in many tumor types, the waters are still muddied in endocrine-dependent tumors and especially in breast cancer. There are a number of studies on both sides of the controversy that show autophagy can both promote and inhibit tumor initiation, tumor progression, response to treatment, and metastasis (Figs. 2 and 3). Compared with the number of comprehensive and in-depth mechanistic studies performed in other tumor types, the field has trailed in endocrine-dependent tumors with far fewer published studies. Additionally, and perhaps accordingly, there are far fewer clinical trials completed or in progress in these tumor types (Table 1). The pessimistic view of these studies might lead to the conclusion that autophagy has too many diverse functions in endocrine-dependent tumors and is not an ideal target. While this may in fact be in the case, the optimistic view is that the complexities have yet to be resolved and further mechanistic work as well as preclinical models are needed to fully elucidate the role of autophagy in endocrine-dependent tumors. For example, a more complete understanding of which subgroups of endocrine-dependent tumors are reliant on autophagy could provide better rational for patient selection and therefore improve efficacy for a select group of patients.

Compared with the number of studies investigating autophagy during tumor initiation and progression, there are far less with a focus on metastasis. With some studies indicating prometastatic and antimetastatic effects in different tumor types, a lot of work is still needed to fully understand how autophagy inhibition in patients might affect the growth of metastatic lesions. Additional preclinical models designed to investigate the effects on metastasis are needed. Moreover, clinical trials should be designed to measure the effects of systemic autophagy inhibition on both the primary tumor and metastatic lesions.

Research Strategy

We used pubmed.gov using search terms autophagy, cancer, breast cancer, prostate cancer, endocrine, CQ, HCQ, etc. and prioritized primary research articles published in the last 1-5 years. We also referenced seminal papers from the last 20 years and comprehensive reviews on the broader topics.

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Disclosures

The authors have no conflicts of interest to declare.

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