

Molecular modulation of autophagy: New venture to target resistant cancer stem cells

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Abstract

Autophagy is a highly regulated catabolic process in which superfluous, damaged organelles and other cytoplasmic constituents are delivered to the lysosome for clearance and the generation of macromolecule substrates during basal or stressed conditions. Autophagy is a bimodal process with a context dependent role in the initiation and the development of cancers. For instance, autophagy provides an adaptive response to cancer stem cells to survive metabolic stresses, by influencing disease propagation *via* modulation of essential signaling pathways or by promoting resistance to chemotherapeutics. Autophagy has been implicated in a cross talk with apoptosis. Understanding the complex interactions provides an opportunity to improve cancer therapy and the clinical outcome for the cancer patients. In this review, we provide a comprehensive view on the current knowledge on autophagy and its role in cancer cells with a particular focus on cancer stem cell homeostasis.

Key words: Autophagy; Cancer stem cells; Cancer cells; Cancer therapy; Therapeutic resistance; Cancer metastasis

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Core tip: Cancer stem cells (CSCs) are a distinct subpopulation in the tumor bulk that are highly plastic, and autophagy has been suggested to modulate their stemness and development during cancer progression. Autophagy is a pro-survival mechanism used by cancer cells to provide bioenergetic substrates. Therefore, dissecting the role of autophagy in cancer propagation can theoretically lead to a more efficient cancer treatment via the modulation of autophagy, in combination with chemotherapeutics to sensitize and target CSCs. This review summarizes the divergent role of autophagy in CSCs and cancer cells and attempts to elucidate the molecular mechanisms involved.

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INTRODUCTION

Autophagy (“self-consumption”) is a conserved catabolic process which assists in the clearance of superfluous, damaged organelles and proteins, and contributes in the recycling of the constituents for the maintenance of metabolic homeostasis and as a pro-survival mechanism^[1]. Autophagy is further activated by intrinsic and environmental stressors including nutrient deprivation, oxidative stress, cytokine and growth factor deficiency, hypoxia and exposure to infection^[2,3]. It can be noted that basal autophagy acts as a quality assurance mechanism in cells and as a source of metabolites^[4]. Dysregulation of autophagy is associated with a variety of inflammatory and infectious conditions, as well as neurodegenerative pathologies, ageing and cancer^[5].

Autophagy is a highly regulated mechanism that facilitates the deliverance of cytoplasmic components for lysosomal mediated degradation. There are three distinct forms of autophagy, such as microautophagy, chaperone mediated autophagy (CMA) and macroautophagy. Microautophagy is modulated by the direct sequestration of cytosolic cargo causing engulfment, followed by indentation of the lysosome leading to degradation^[6]. In comparison, CMA is a prime example of selective autophagy. In this particular pathway, chaperones are utilized targeting specific proteins containing a pentapeptide KFERQ motif sequence. Once engaged this leads to the translocation across the lysosome membrane mediated by lysosome associated membrane protein 2A^[7,8]. In contrast, macroautophagy (herein referred to as autophagy) initiates the degradation of intracellular organelles by delivering them to the lysosome by sequestering sections of the cytoplasm via double membrane vesicles called autophagosomes. The fusion between these two entities not only promotes degradation but also generates bioenergetic substances for recycling. Emerging studies describe the existence of a cross talk between CMA and macroautophagy that promotes a compensatory mechanism under basal and stressed conditions^[7,9].

The regulation and process of canonical autophagy

Autophagy relates genes (*Atg*) are involved in the development and turnover of the autophagosomes. Formation of the autophagosome proceeds through multiple steps that include initiation, nucleation, elongation, maturation and thereafter fusion with the lysosome^[8,10]. The mitochondria and the endoplasmic reticulum (ER) are contact sites for the formation of autophagosomes^[11,12] (Figure 1).

Upstream signaling pathway such mammalian target of rapamycin (mTOR) is a major negative regulator of autophagy as it senses amino acid availability and initiation of cellular anabolism. 5'-AMP-activated protein kinase (AMPK) is activated during starvation^[2]. Under these circumstances, AMPK phosphorylates tuber sclerosis complex 2 which inhibits mTOR^[13]. Moreover, Atg7 protein is essential in modulating starvation-induced autophagy as demonstrated in *Atg7* conditional knockout mice^[14].

The inhibition of mTOR sequentially leads to the activation of pre initiation complex composed of unc-51-like kinase 1 (ULK1) complex, FAK family kinase interacting protein of 200 kDa, Atg13 and Atg101, causing translocation to the membrane, and triggering the initiation step for the assembly of autophagosomes^[10]. The ULK1 complex phosphorylates the class III phosphatidylinositol-3-kinase (PI3K) vacuole protein sorting 34 (VPS34) complex; consisting of VPS15, Beclin-1 (BECN1) and Atg14, which stimulates the generation of phosphatidylinositol-3-phosphate 3 (PI3P), an essential lipid molecule required for the nucleation step of the phagophore^[15-17]. Atg9 positive vesicles on the ER contribute to the nucleation process by interacting with the ULK1 complex^[17]. To promote autophagosomes elongation, WD repeat domain phosphoinositide-interacting protein 2 (WIPI-2) and zinc-finger FYVE domain-containing protein 1 are employed for the recruitment of two ubiquitin like systems^[16]. Firstly, Atg7 and Atg10 act as E1 like and E2 like enzymes to covalently conjugate Atg12 to Atg5 and then attach to Atg16L^[8,18,19]. In the second conjugation pathway, Atg12-Atg5 conjugate serves as an E3 like enzyme, where Atg8 family member LC3 is attached to phosphatidylethanolamine^[2,19]. Atg7 and Atg3 mediate this process. Next, the autophagosome matures by membrane bound LC3.

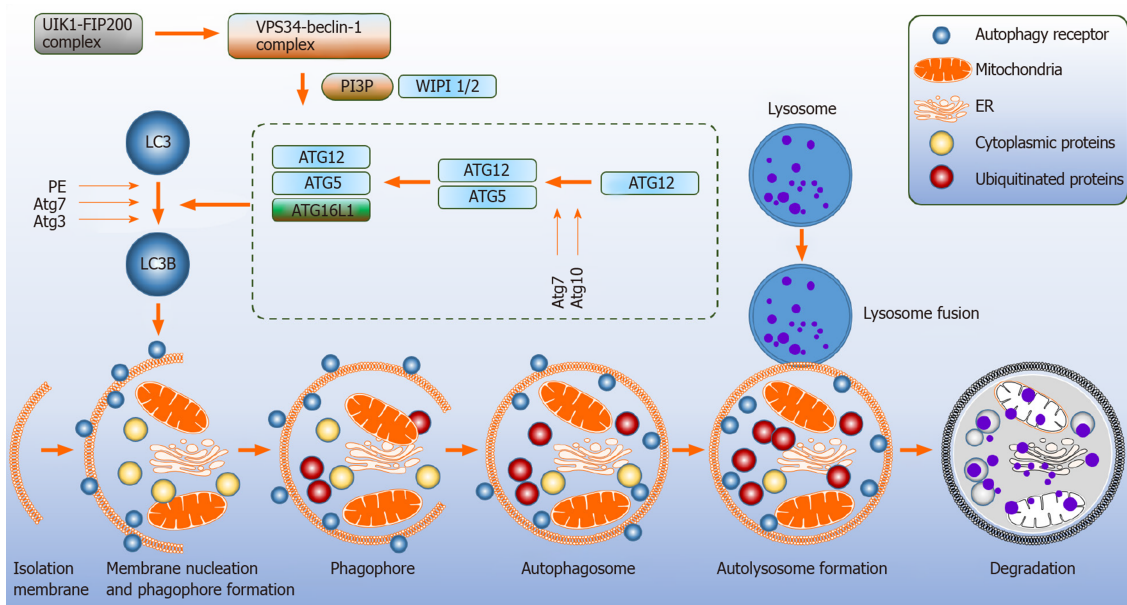


Figure 1 Canonical autophagy pathway. Autophagy is a multistep process that includes the following steps: initiation, nucleation, elongation, maturation and fusion with the lysosome. Several proteins referred to as autophagy related genes regulate this process. Autophagy is stimulated under basal conditions and is induced by stress, for example nutrient deprivation. ATG: Autophagy related genes; ER: Endothelial reticulum; FIP200: FAK family kinase interacting protein of 200 kDa; LC3: Light chain 3; PE: Phosphatidylethanolamine; PI3P: Phosphatidylinositol-3-phosphate 3; ULK1: Unc-51-like kinase 1; VPS34: Vacuole protein sorting 34.

NBR1 neighbor of BRAC1 and adaptor protein p62 facilitate in the degradation of misfolded and ubiquitinated substrates by binding to Atg8-LC3^[18-20]. The closure of the autophagosome is driven by LC3 causing the Atg12-Atg5-Atg16L complex to dissociate from the autophagosome membrane leaving the lipidated LC3 (LC3B; microtubule-associated proteins 1A/1B light chain 3B) in the autophagosome^[16,18]. The degradation of LC3B and p62 are widely accepted markers to measure the autophagic flux.

It should be noted, however, that multiple signaling cascades control autophagy and modify ULK1 and class III PI3K complexes. These include antigen specific receptors (B cell receptor and T cell receptor), CD40 “the co-stimulatory molecule”, Toll like receptors, cytokine receptors and nucleotide-binding oligomerization domain protein 2^[2]. The VPS34-BECN1 complex can be inactivated by the anti-apoptotic proteins from the B cell lymphoma-2 (BCL-2) family^[16]. Here we have discussed the major canonical pathway that utilizes mTOR (Figure 1).

Non-canonical autophagy

Autophagy that precedes the formation of autophagosomes without the involvement of the core machinery is referred to as non-canonical autophagy. An example of non-canonical autophagy would be LC3-associated phagocytosis (LAP) which depends on class III PI3K subunit called RUBICON, a negative regulator of autophagy^[2,21]. Unlike canonical autophagy, LAP only requires BECN1 and VPS34 as a pre-initiation complex and downstream conjugation of LC3 to generate NADH oxidase 2^[22]. LAP-LC3 is associated to autophagosome maturation and facilitating the degradation of engulfed cells. LAP does not respond to nutrient deficiency or intracellular stressors, unlike canonical autophagy. Additionally, the substrates for this process are extracellular entities including Toll like receptor, pattern recognition receptors and dead cells^[22]. LAP occurs in multiple immune cells, such as macrophages, dendritic cells (DCs) and epithelial cells^[21]. LAP deficiency in cells and animal models trigger exaggerated inflammation^[22].

In the canonical form, it is assumed that the generation of PI3P is essential for the process of autophagy. However, Mauthe *et al*^[23] reported resveratrol mediated autophagy did not stimulate PI3P dependent accumulation of WIPI-1 at the autophagosome membrane. This finding was confirmed by PI3P inhibition using wortmannin in combination with resveratrol which led to an increased autophagic flux of LC3B and GFP-LC3 puncta formation. This was promoted in the absence of phagophore formation suggesting an alternative contact site for autophagosome formation. Additionally, the actions of resveratrol were found to be independent of BECN1; however, required Atg7 and Atg5 to induce the LC3 lipidation. It can be concluded that resveratrol induces non-canonical autophagy^[23].

The origin of the autophagosome membrane and the formation of the autophagosome remains unclear^[24]. Recently, using freeze fracture replica immunolabelling, WIPI-1 puncta were found to be localized on the ER and Plasma membrane and WIPI-2 was detected close to the Golgi cisternae under starvation induced autophagy, exclusively. These findings suggest that WIPI-1 and WIPI-2 are essential components of the autophagosome and the autophagosome membrane site and formation may potentially originate from the ER, Plasma membrane and the Golgi^[25]. Interestingly, the deletion of WIPI-2 in the germinal center (GC) B cells enhanced the autophagic activity, suggesting that B cells derived from the GC have the ability to switch from canonical autophagy upon challenge to non-canonical autophagy to meet their metabolic demands^[26].

It is believed that Atg5 and Atg7 are essential for autophagy. However, recent studies have challenged this notion. Atg5/Atg7 independent non-canonical autophagic pathway have been identified, which are able to form autophagosomes mediated in a Rab9 dependent manner from the trans-Golgi network and late endosomes. Autophagy proteins, such as ULK1 and BECN1 were found to regulate this process independent of LC3^[27]. The resulting autophagosomes mature and fuse with the lysosome and undergo cargo clearance^[28]. Furthermore, ULK1 dependent/Atg5 independent autophagy has been implicated in the removal of the mitochondria from fetal definitive erythroid cells *in vivo*^[29]. Additionally, *ULK1*^{-/-} mice models were able to express LC3B under nutrient depleted conditions; indicating the role of ULK1 in the induction of autophagy is dispensable^[30]. These reported studies suggest ULK1 is not essential for Atg5/Atg7 dependent canonical autophagy^[14,31]. Moreover, ULK1 is upregulated during non-canonical autophagy, and the silencing of *ULK1* inhibits this process^[27].

Cross talk between autophagy and apoptosis

It is evident that autophagy participates in catabolism including the breakdown of long-lived proteins, providing bioenergetics material to facilitate in the production of adenosine triphosphate (ATP) and meet the metabolic demands of cells undergoing adverse conditions and rescue them. However, under prolonged metabolic-stressed conditions the pool of bioenergetic substrates will be facilitated to generate ATP dependent apoptosis^[32]. Predominately, autophagy has a cytoprotective role. Overall, it can be assumed that autophagy and apoptosis are activated by a common stimulus^[19].

Apoptosis “self-killing” is a form of type 1 programmed cell death (PD) and is characterized by the distinct morphological changes causing nuclear condensation (Pyknosis) and fragmentation (Karyorhexis), and membrane blebbing a requisite for the generation of apoptotic bodies (smaller apoptotic cell fragments)^[33,34].

Emerging literature indicates a complex network that regulates the interplay between autophagy and apoptosis. This is cell type and stimuli dependent. This dynamic interplay has been described in the following examples: Autophagy and apoptosis can function together in order to induce cell death, autophagy can promote cell survival by antagonizing apoptosis, or autophagy can assist in cell death by activating apoptosis^[16,35,36].

Multiple stimuli that can trigger cell death can also induce autophagy. Autophagy as a cytoprotective mechanism is usually induced first, followed by apoptosis^[16,37]. Death associated protein kinase (DAPK) signaling is an example when both apoptosis and autophagy are induced either simultaneously or sequentially. Upon stimulation, DAPK phosphorylates BECN1 leading to its dissociation from BCL-2; thus, activating autophagy by binding to VPS34^[38,39]. However, activated DAPK is also able to stimulate apoptosis in autophagy deficient conditions^[40]. It can be postulated that DAPK regulated autophagy is induced by low levels of stress, however, intense and chronic stress stimuli can initiate apoptosis through DAPK^[16].

It has been proposed that autophagy and apoptosis display an inhibitory relationship during the removal of pro-apoptotic proteins in the cytoplasm caused by autophagy, resulting in reduced apoptosis. Caspase-8 activation is a critical step during the extrinsic apoptosis signaling. However, selective autophagy may interfere in an inhibitory manner with the cell-death pathway through the degradation of caspase-8^[41]. Furthermore, autophagy can be inhibited by apoptosis *via* numerous mechanisms; for example, autophagy exhaustion during increased intensity levels of stress. In this condition, degradation of autophagic proteins and caspases activity is reduced. For example, BECN1 inactivation occurs after caspase-mediated cleavage, stimulating the release of pro-apoptotic factors, and resulting in autophagy inhibition and induction of apoptosis^[42].

Autophagy dependent cell death is defined as a form of cell death distinct from apoptosis or necrosis that mechanistically depends on the autophagic machinery^[43]. It is postulated that the formation of autophagosome, and not degradation, leads to the

activation of caspase-8 and the execution of cell death. As reported in mouse embryonic fibroblasts treated with proteasome inhibitor Bortezomib, and pan-sphingosine kinase inhibitor. Pro-caspase-8 interactions with p62 have been shown to co-localize with the autophagosomes. The surface of the autophagosomes serves as a platform for the maturation of caspase-8 and the initiation of apoptosis^[16,43]. Furthermore, the depletion of *Atg5* ablated caspase-8 processing in the presence of Bortezomib leading to a significant reduction in cell death^[44].

CANCER STEM CELLS

Tumors are derived from heterogeneous cell types. Cancer stem cells (CSCs; also known as tumor initiating cells) are a small subpopulation of cancer cells within the tumor bulk tissue that retain the capacity for self-renewal, disease propagation, and metastasis, which are decisive for tumor recurrences and are therapy resistance^[45-47]. In general, stem cells are characterized by their distinct ability to switch their cell cycle profile from quiescent to proliferative behavior in order to maintain their capability for self-renewal and later multi-potency^[48]. Similarly, CSCs have the extraordinary capability to self-renew and differentiate rapidly; accumulating mutations and genetic alterations and transmitting these defects to the proliferating progeny, giving rise to tumor heterogeneity conferring to resistance against anti-cancer therapeutics^[49].

Similar to normal stem cells, CSCs reside in dynamic microenvironments known as the stem cell niche, this regulates the fate of adult stem cells by providing signals, such as cell-cell contact and secreting mediators to promote CSCs renewal, tumor invasion and metastasis^[24,50]. Normal niches are comprised of heterogeneous collection of cells, such as endothelial, fibroblasts, immune cells, perivascular cells, components of the extracellular matrix, cytokines and growth factors^[51]. In comparison, the CSCs niche itself is part of the tumor specific microenvironment that remains distinct from the normal niche^[52]. During tumor progression to a malignancy, the CSC state in the primary tumor depends crucially on the microenvironment and potentially on the CSC niche itself^[53]. Targeting the CSCs niche is the current subject of research as it is a valuable modality for the treatment and prevention of CSCs growth and downstream signaling^[52].

The functional characterization of CSCs in multiple studies have clarified that CSCs are the foundation of tumor formation that can survive treatment with conventional therapies and can cause the recurrence of cancer^[54,55]. According to the concept of a stem cell, it can be assumed that even a few surviving CSCs after therapy is sufficient to develop a new tumor leading to a relapse. Due to the ability of CSCs to initiate relapse after conventional cancer therapy, they represent a crucial therapeutic target^[46]. CSCs were first identified in acute myeloid leukemia (AML); their presence was confirmed by the isolation of AML-initiating cells based on their phenotypical markers^[56]. In solid tumors, breast cancer was one of the first to be characterized, which led to the identification of a specific subpopulation of CSCs marked by CD44⁺CD24^{-/Low} lineage. This tumorigenic population of cells was able to initiate tumor growth in immunosuppressed mice^[57,58]. Furthermore, CSCs have been discovered in several solid cancers, such as lung^[55], pancreatic^[59], colon^[60,61], melanoma^[62], ovarian^[63,64], brain cancers^[65,66] and hematological malignancies of both myeloid and lymphoid origin^[67-69].

CSC models of tumorigenesis and plasticity

To date, two paradigms: hierarchical and stochastic have been proposed to account for the tumor origin, progression and heterogeneity. In brief, the hierarchical model is based on a concept that tumor cells are hierarchically arranged cell populations and CSCs represent the top of the arrangement. Carcinogenesis proceeds when a healthy normal stem cell escapes regulation and transforms into a stem cell-like phenotype-CSCs. This in turn gives rise to heterogeneity by generating differentiated and quiescent cells whose proliferation capacity is restricted^[52,70]. By contrast, in the stochastic model, cancer is derived from a single somatic cell that initiates tumorigenesis and progression. This paradigm partially relies upon the environment in which the cancer cell is located in, but, fundamentally is defined by hyper proliferation and the acquisition of mutational burden during the cell cycle process contributing to clonal expansion^[52].

AUTOPHAGY IN CANCER STEM CELLS

Role of autophagy in the maintenance of CSCs

As highlighted earlier in this review, autophagy is a multifaceted pro-survival mechanism. In cancer, the role of autophagy is context dependent. Autophagy elicits tumor suppressing functions during tumor initiation by limiting inflammation, tissue damage, and genome instability by removing damaged mitochondria and reducing oxidative stress^[2]. Extracellular stimuli, such as oxidative stress, nutrient depletion, increased metabolism and hypoxia result to disease propagation; thus, demanding autophagy to meet the high metabolic demands by providing recycled bioenergetic substrates to the CSCs, and whilst doing so, implementing its role as a tumor promotor (Figure 2)^[71].

It has been proposed that autophagy is associated to CSC maintenance. *LC3B* gene knockdown in human embryonic stem cells (ESCs) leads to a reduction in pluripotency and due to the accumulation of pluripotency associated proteins suggesting autophagy regulates these proteins^[72]. Autophagic flux is upregulated in mammospheres in basal and starvation-induced autophagy and is driven by *BECN1* and *Atg4A* for their survival and expansion. Inhibition of these autophagy genes abolishes the tumor formation^[73,74]. Aldehyde dehydrogenase 1-positive (ALDH1⁺) CSCs isolated from MCF-7 mammospheres presented an increased LC3B dependent autophagic flux with higher rate of p62 degradation compared to the bulk population; indicating an increased synthesis of autophagosomes. In addition, suggesting elevated autophagy is critical for CSCs^[74]. Moreover, Antonelli *et al.*^[75] reports that ataxia-telangiectasia mutated (ATM) kinase modulates breast CSCs through *Atg4C*. This was validated in an overexpression study of *Atg4C* that was assessed in *ATM* gene silenced cells using *shATM*; this led to the rescue of mammosphere formation in *ATM* knockdown cells. These findings correlated with the microarray data of breast cancer samples, however, excluded triple negative tumors^[75]. Indeed, these autophagy genes have shown to promote CSC survival and tumorigenicity. RNAi screenings reveal constitutive STAT3 activity is regulated by autophagy and is enriched in the triple negative breast cancer cell lines^[73,76]. In those cell lines, *Atg7* and *BECN1* modulate CD24 expression in CD44⁺CD24^{-/low} CSC population and secret interleukin 6 (IL-6) through gp130 and JAK-STAT pathway for CSC maintenance^[73,77].

MMTV-PyMT is a well-characterized transgenic murine model for breast CSCs tumorigenesis. Yeo *et al.*^[78] reported autophagy differentially regulates two distinct breast cancer stem-like cells *ALDH1*⁺ and CD29^{hi}CD61⁺ though EGFR/STAT3 and Tgfβ/Smad. Depletion of *FIP200* decreased STAT3 activation by decreasing phosphorylation of EGFR and had consequently impaired the tumor initiating properties of *ALDH1*⁺ and CD29^{high}CD61⁺ breast CSCs. Autophagy inhibition led to decreased mRNA levels of *TGFβ2* and *TGFβ3* triggering dysregulation in Smad signaling which is essential for CD29^{high}CD61⁺ CSCs^[78]. The secretion of IL-6 is autophagy dependent and is mediated through STAT3/JAK2 pathway^[77]. From these studies, it can be assumed STAT3 signaling may potentially be an important factor in CSCs transformation.

In general, FOXO transcription factors have been associated in the regulation of cellular homeostasis, stem cell maintenance, ageing and tumor suppression. Mice with somatic deletion of *FOXO1*, *FOXO2* and *FOXO4* resulted in thymic lymphomas and hemangiomas^[79]. Upregulation of *FOXO1* promotes self-renewal of t(8;21) pre leukemia cells *in vitro* and *in vivo*, and restricts differentiation of AML cells with t(8;21) translocation; indicating *FOXO1* is not a tumor suppressor, however, plays a crucial role in leukemia stem cells (LSCs) maintenance^[80]. Absence of *FOXO3* has been reported to contribute to the expansion of CSC population as well as increase self-renewal and tumorigenesis in prostate^[81], colon^[82], and glioblastoma^[83] and promote tumor initiation in breast cancer^[84]. Recently, it has been proposed that DNA methyltransferase 1 mediates *FOXO3a* promoter hyper methylation causing downregulation of *FOXO3a* gene expression in breast CSCs; thus, suppressing CSCs phenotype markers and tumorigenicity^[85]. To date, the role of FOXO in CSCs remains controversial. It has been reported FOXOs are implicated in autophagy^[86-88]. *FOXO3* overexpression studies reveal this gene directly regulates autophagy related genes involved in the autophagosome pre-initiation complex: *WIPI-1/2*, core initiation complex: *ULK1*, autophagosome formation and elongation: *Atg14*, *GABARAP*, *Atg5*, *Atg10*. *FOXO3* knockout cells downregulated many of these genes and *PINK1* (component of mitophagy) and exhibited poor LC3B lipidation turnover; indicating *FOXOs* are required to maintain basal autophagy in neural stem and progenitor cells^[87]. *FOXO3A* induced autophagy promotes survival in human pluripotent stem cells^[88]. The pro autophagy protein, *AMBRA1*, modulates the differentiation of regulatory T cells through *FOXO3/FOXP3* axis. In the context of immunosurveillance against tumors, *AMBRA1* deficiency leads to defective generation of the induced

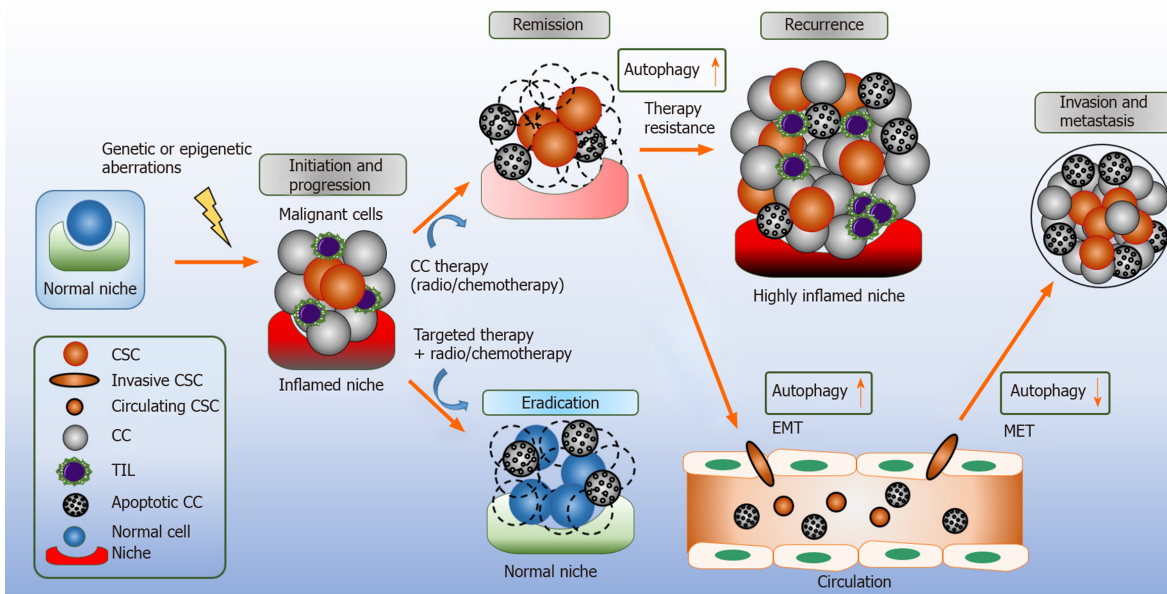


Figure 2 Autophagy in cancer stem cells. Autophagy has a context dependent role in cancer. Cancer stem cells (CSCs) are a heterogeneous collection of different cells types that acquire genetic aberrations/epigenetic modifications and retain the ability to undergo extensive cell proliferation, retain stemness and give rise to differentiated diverse cancer cell lineages. Potentially the CSC niche will provide protective mechanisms for the disease propagation. Autophagy promotes invasion of cancer stem cells through TGF- β dependent epithelial-mesenchymal transition; however, during mesenchymal-epithelial transition autophagy is downregulated as the circulating CSCs are scavenging an organ to seed for metastasis. Moreover, autophagy reinforces the resilience of CSCs plasticity, remodeling the immunosurveillance and facilitating the acquisition of resistance to conventional chemotherapies which contribute to cancer relapse. By targeting autophagy, cancer cells and CSCs are sensitized to enhancing the efficacy of chemotherapy agents and reducing their toxicity and disease relapse. CSC: Cancer stem cell; CC: Cancer cell; EMT: Epithelial-mesenchymal transition; MET: Mesenchymal-epithelial transition; TIL: Tumor-infiltrating lymphocytes.

regulatory T cells in lymph nodes of tumor bearing mice; influencing the regulatory T cells function in tumor response^[89].

Additional FOXO family members are associated to autophagy. It is reported that *FOXA2* knockdown in ovarian CSCs leads to a reduction in the number of spherical clusters of cells, size and the percentage of phenotype surface markers; suggesting *FOX2A* modulates the ability of self-renewal *in vitro*^[90]. Inhibition of autophagy by *Atg5* knockdown, bafilomycin A1 (vacuolar H⁺ ATPase inhibitor) or chloroquine (CQ; lysosomotropic agent- late stage autophagy inhibitor) repressed *FOXA2* expression. *FOXA2* overexpression partially rescues these effects; indicating autophagy modulates ovarian CSCs stemness through *FOX2A*^[90]. These studies identify a synergy between FOXOs and autophagy. This relationship promotes CSC stemness and tumorigenesis; however, the mechanisms behind these actions remains unclear and require further elucidation. Though it is noteworthy that the regulatory role of autophagy in CSC is very complex.

Autophagy induces metabolic reprogramming in CSCs

The tumor microenvironment (TME) is a critical driver of tumor heterogeneity, encouraging CSCs plasticity, remodeling immune surveillance, and facilitating their metastatic potential and ultimately conferring CSCs resistance to chemotherapy drugs^[52,91]. Non neoplastic cells, and their secreted mediators, such as growth factors and the release of cytokines, are found to contribute to the TME^[92]. The core regulatory mechanism for oxygen sensing and adaption to hypoxia is hypoxia inducible factor (HIF), in particular HIF-1 α and HIF-2 α . HIF target genes are able to induce human ESCs markers sufficient to induce pluripotent stem cell inducers: *OCT4*, *NANOG*, *SOX2*, *KLF4*, *MYC* and *miRNA-302* in multiple cancer cell lines. Similar results were reported in prostate tumors^[93]. Hypoxia-Notch1-SOX2 signaling axis has been found to activate ovarian CSCs by stimulating self-renewal capacity and drug resistance^[94]. Hypoxia activation and upregulation of HIFs has been implicated in aggressive tumor phenotypes, including breast and glioma CSCs, as a result correlating with co-localization studies of these markers with CSCs markers results to poor survival outcome in cancer patients^[95,96].

The integration of autophagy in the cancer stem niche provides metabolic plasticity to CSCs from hypoxic conditions, nutrient limitation and acidosis^[92]. Immunohistochemistry of pancreatic ductal adenocarcinoma (PDA) tissues reveals co-expression of hypoxia, pancreatic CSC markers (CD44, CD24) and autophagy (BECN1

and LC3B). Hypoxia starvation induced autophagy has been demonstrated to increase clonogenicity and migration of PDA-CSC^{High} cells and the number of autophagosomes formed^[97]. In accordance, CD133⁺ pancreatic CSCs is dependent on HIF-1 α to induce autophagy for stem cell maintenance^[98]. Similarly, CD133⁺ liver CSCs showed higher survival capacity under hypoxic and nutrient deprived conditions^[99]. Recently, phosphorylation of EZR at Thr567 residue and activation of PRKCA/PKC α kinase has been suggested to be a responsible candidate for enhanced self-renewal capacity of colorectal CSCs in hypoxia induced autophagy. The blockade of *Atg5*, *BNIP3*, *BNIP3L*, or *BECN1* reversed these effects^[100]. Limited literature is available to define the exact interplay between hypoxia, autophagy and the maintenance of the TME.

HIF-1 α enhances the secretion of TGF- β 1-Smad in mesenchymal stem cells (MSC) which facilitates the propagation of CD44⁺ breast cancer stem-like cells^[94], promoting epithelial to mesenchymal transition (EMT)^[101]. Autophagy inhibition by *Atg5* silencing and CQ treatment, notably enhanced the transcriptional activation of epithelial marker CD24 whilst repressing EMT marker vimentin in response to TGF- β 1, dysregulating cellular ability to migrate and invade^[102]. In non small cell lung cells, vimentin was downregulated in the presence of TGF- β 1 treatment in *Atg7* knockdown cells, indicating autophagy positively regulates TGF- β 1 in EMT^[103]. To the contrary, autophagic targeting of EMT transcription factors, such as *Snail* and *TWIST*, through death-effector domain-containing DNA-binding protein-PI3KC3 has been shown to inhibit tumor metastasis and growth in breast cancer^[104]. The divergent role of autophagy in EMT has been illustrated in **Figure 2**.

Recently, it has been shown that pluripotent transcription factor NANOG, contributes to hypoxia-induced autophagy by directly activating *BNIP3L*. NANOG promotes resistance to immune mediated actions of cytotoxic T cells^[105].

Mitophagy is the selective degradation of defective mitochondria by autophagy to avoid the accumulation of oxygen species and its association to cell death, senescence and malignant transformation. Mitochondria has a central role in generating ATP derived from oxidative phosphorylation (OXPHOS) and the tricarboxylic acid cycle^[2]. Human pancreatic CSCs are primarily reliant upon OXPHOS for energy acquisition, as compared to their counterpart; indicating increased mitochondrial activity contributes to CSC stemness^[106]. Similar results were observed in mice exhibiting *KRAS* gene ablation in pancreatic adenocarcinoma cells^[107]. Moreover, *KRASG12D* mutated pancreatic adenocarcinoma cells have been shown to enter into quiescence in response to oncogene ablation and did not present metabolic stress and induced autophagy. This finding was confirmed by measuring the levels of LC3B by immunoblotting and using flow cytometry to quantify the autophagic flux of *KRAS* mutated cells stably expressing GFP-LC3; and Bafilomycin A1 treatment rescued the GFP signaling. Interestingly, these cancer cells exhibited stem cell-like phenotype^[107]. Increased mitophagy is reported in esophageal squamous cell carcinoma CD44^{High} undergoing EMT; the expression of CD44 is rendered during the inhibition of Parkin dependent mitophagy, resulting to cell death^[108]. Hepatic CSCs stemness and self-renewal capacity is maintained by the removal of p53 localized to the mitochondria and removed in a mitophagy dependent manner. In contrast, during the suppression of mitophagy, p53 is phosphorylated by PINK1 and translocated to the nucleus to prevent *Oct4*, *SOX2* and *NANOG* transcription in the hepatic CSC population. These results suggest that the activity of p53 is regulated by mitophagy to promote hepatocarcinogenesis^[94]. In LSCs, the loss of p53 simultaneously activates endogenous *KRASG12D* mutation inducing aggressive AML phenotype; thus, enabling abnormal growth^[109]. Mitophagy is activated in LSCs by the constitutive activity of AMPK and FIS1; preventing differentiation via GSK3 downstream mechanism and promotes stemness. Inhibition of AMPK-FIS1 axis results to suppression of proliferation and induction of differentiation^[110].

THE ROLE OF AUTOPHAGY IN DIFFERENTIATED CANCER CELLS

Bcl-2 binds directly to *BECN1* and plays a vital role in the development and differentiation of normal B cells to inhibit autophagy^[111-113]. In accordance, immunohistochemistry studies of patients with diffuse large B cell lymphomas (DLBCL) revealed that increased *BECN1* levels with reduced levels of Bcl-2 correlated favorably to the clinical survival outcome with better response to the first line treatment of R-CHOP^[114,115]. The incidence of breast, ovarian and prostate cancer is higher in 40%-75% patients with monoallelic deletions of *BECN1* gene. Furthermore, in mice with heterozygous deletion of *BECN1* predisposed them to spontaneous malignancies including DLBCL, suggesting *BECN1* is a haplo-insufficient tumor

suppressor gene^[116]. Similar findings were reported in the incidence of pre-B acute lymphoblastic lymphoma with elevated expressions of programmed death ligand 1 (PD-L1) and IL-10^[117]. A study led by Bertolo *et al*^[118], suggests constitutive suppression of autophagy responses in BCL-6 driven GC-derived lymphomas, including DLBCL contribute to lymphomagenesis. In mice, the homozygote deletion of *BECN1* results to embryonic lethality, in comparison *BECN1* heterozygous deletion leads to the establishment of spontaneous tumors and defective autophagy; however, did not impair apoptosis^[119].

Enhanced autophagic flux has been attributed significantly to metastatic tumorigenesis and immunosuppression related chemoresistance. In *ex vivo* lung cancer cells, CQ augments carboplatin treatment by sensitizing the lung cancer drug resistant cells and non-resistant cells by limiting the proliferation status and providing synergistic effects with carboplatin to induce apoptosis. These findings corroborated with the decreased LC3B level and BECN1 protein expressions suggesting a decrease in the formation of autophagosomes. The administration of CQ in drug resistant cancer cells, strikingly reduced the drug resistant proteins: MDR1, MRP1 and ABCG2 and mRNA reduction of *MRP1* and *ABCC2*. The combination treatment of CQ and Carboplatin significantly reduced both the protein and the mRNA levels. Furthermore, this decreased the expression of PD-L1 suggesting autophagy has a role in modulating of PD-L1 in cancer evasion and immunosuppression. Interestingly, the combination treatment promoted the infiltration of CD4⁺, FOXP3⁺ tumor infiltrating lymphocytes (TILs) indicating autophagy inhibition with carboplatin could mediate lymphocyte infiltration in the tumor and upregulate only specific expression of TILs, leading to immune system activation^[120].

THE ROLE OF AUTOPHAGY IN TUMOR DORMANCY

Cancer progression leads to metastatic growth resulting to a majority of cancer related deaths^[121]. In many cases, dissemination of tumor cells (DTCs) has already occurred in patients at diagnosis. It is challenging to detect DTCs at secondary sites, as they may have entered into dormancy and become refractory to therapeutic targets^[122]. The divergent characteristics of DTCs have emphasized the need to improve this phenomenon. It is postulated that autophagy is activated during the seeding process of DTCs at secondary sites providing an adaptive response to nutrient depletion and environmental stress^[123]. For example, the tumor suppressor gene *ARHI* (RAS homologue) is downregulated in 60% of ovarian cancer cases. Studies in ovarian cancer cell lines revealed autophagy induction is mediated by *ARHI* as it inhibits PI3K-mTOR signaling. This is corroborated by co-localization staining of Atg4 and LC3B in autophagosomes suggesting *ARHI* facilitates the autophagosome formation through this signaling. Xenograft model expressing SKOV3-*ARHI* cells supplemented with *ARHI* by doxycycline repressed tumor growth, however, the withdrawal of *ARHI* after 32 or 42 d stimulated rapid tumor growth, indicating that the cancer cells, in particular, CSCs remained viable and dormant during latency. Autophagy inhibition by CQ in this model confirms dormancy requires *ARHI* mediated autophagy^[124]. Accordingly, *Atg7* is essential for the reduction of lung metastatic burden utilizing a non-canonical autophagy pathway independent of *BECN1*^[125]. In contrast, recent microarray analysis of CSCs in breast cancer patients revealed the expression of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*Pfkfb3*), which correlated with an aggressive cancer phenotype coupled with self-renewal ability and metastasis potential. Dormant breast cancer cells display *Pfkfb3*^{Low}Autophagy^{High} phenotype with elevated levels of LC3B and p62. In contrast, the metastatic breast cancer cells which exhibited *Pfkfb3*^{High}Autophagy^{Low}; suggesting the status of autophagy changes during the phenotypic transition. Knockdown of *Atg3*, *Atg7* or *p62* genes promoted the proliferation and outgrowth restoring the upregulated expression of *Pfkfb3* in dormant breast CSCs. The ablation of autophagy related genes gained CD49^{High}/CD24^{Low} phenotype with increased stemness signature in CSCs. These findings reveal autophagy activation could function to prolong the overall survival of patients by promoting permanent dormancy of CSCs. Additionally, *Pfkfb3* protein was found to directly interact with ubiquitin binding domain of p62, suggesting its role as a substrate. Moreover, inactivation of autophagy can facilitate dormancy of breast CSCs to metastatic lesions by stabilizing *Pfkfb3* gene expressions *via* p62^[126]. These studies highlight the poorly understood role of autophagy during dormancy in breast CSCs, in which targeting autophagy enables the sensitization of CSCs to chemotherapy by eliminating the adaptive response to autophagy^[124,125]. Though it is noteworthy, CSCs are heterogeneous and disease specificity adds complexity to the matter^[126].

CLINICAL IMPLICATIONS OF TARGETING AUTOPHAGY IN RESISTANCE CANCER STEM CELLS

Autophagy demonstrates tumor-suppressing actions in early cancer initiation; but fundamentally provides adaptive responses – an advantage to cancer cells and CSC during cellular stress. It remains an open question whether to stimulate or to inhibit autophagy in cancer, specifically, in combination with anti-cancer therapeutics. Autophagy inhibition may provide a reasonable rationale to be used; as multiple tumors stimulate autophagy as a source of nutrient replenishment for their increased metabolic demands, survival and disease propagation (Figure 3)^[127]. Hydroxychloroquine (HCQ) is an FDA approved drug, with the capability to suppress autophagy at a later stage by inhibiting lysosome acidification and due to these functions it has been used in numerous early phase clinical trials^[128]. Meta-analysis data reveals autophagy inhibition based treatment leads to a better therapeutic response as compared to chemotherapy or radiation in the absence of autophagy; suggesting this may provide a new therapeutic strategy for anti-tumoral therapy^[129]. However, the activation of autophagy may potentially hold a beneficial role as an anticancer therapy. For example, tat-BECN1 peptide was shown to induce autophagy in *HER2*-positive breast cancer xenografts and prevented tumor growth^[131].

In hindsight, CSCs and their counterparts have a unique and complex interaction within the tumor niche which challenges the opportunity to target autophagy directly^[132]. Several studies indicate the beneficial impact of combination treatments of chemotherapeutics with autophagy modulators. For instance, the combination of autophagy modulators with chemotherapy showed to stimulate of CD8⁺ T cell-dependent anticancer immune responses leading to tumor sensitization and cancer cell growth reduction (Figure 2)^[133].

TARGETING CANCER CELLS AND CANCER STEM CELLS USING AUTOPHAGY MODULATORS

CSCs are highly tumorigenic and contribute to cancer relapse due to their ability to self-renew and differentiate into heterogeneous cancer cell lineages. Their resilience is demonstrated in the treatment of chemotherapy and radiation therapy^[57]. In addition, CSCs are able to remain in a quiescent state and cultivate their ability to become resistant by gaining adaptation to their environment^[123]. For example, in castration resistant prostate cancer it has been shown that autophagy is induced during Docetaxel treatment and STAT3 contributes to cancer cell survival^[134]; suggesting it is important to target autophagy directly or as a combination treatment to sensitize cancer cells.

It should be highlighted that CQ and HCQ exert anti-tumor effects in combination with anti-cancer treatments in clinical trials^[135]. In PDA the combination of Gemcitabine with HCQ was assessed^[136], this was also evaluated in studies of breast cancer and irradiation^[137], or in combination with the autophagy inducer Temsirolimus in patients with various solid cancers including melanoma^[138]. In preclinical *in vitro* models of breast cancer, similar results were reported^[139]. These findings suggest that autophagy inhibition and activation are promising methods to elicit the sensitization of CSCs to chemotherapy. Moreover, it can be concluded that metastatic cells are preferentially vulnerable to lysosomal inhibition; however, it would be important to assess if these metastatic cancer cells express stem cell-like phenotypical features^[140]. For example, autophagy inhibition in breast CSCs expressing Pfkfb3 were found to promote tumor metastasis^[126]; suggesting therapeutic strategies involving autophagy modulation in treating CSCs, also depends on the cancer phenotype. As mentioned above, CQ and HCQ have been used as late stage autophagy inhibitors in numerous studies. However, the development of newer generation of lysosome inhibitors are more selective and potent which have been introduced, including Lys05 (analogue of CQ) and dimeric quinacrine (DQ661) - a derivative of Lys05. Both are specific in targeting the lysosome and causing impairment of palmitoyl-protein thioesterase activity by impairing mTOR signaling pathway^[141]. Lys05 is a potent autophagy inhibitor in comparison to HCQ. Lys05 has shown to decrease the number of LSCs *in vitro* by promoting their maturation; similar results were seen in patient-derived samples^[142]. DQ661 is effective in targeting cancer paradigms of melanoma, colon cancer and PDA by repressing growth and inhibiting autophagy^[141]. Inhibitor of V-ATPase called Concanamycin A, protease inhibitor E64d and pepstatin A, have also been introduced^[143]. These autophagy modulators are providing an opportunity to explore different combination treatments in different cancer types. Moreover, these lysosomotropic targets are deemed to be effective in

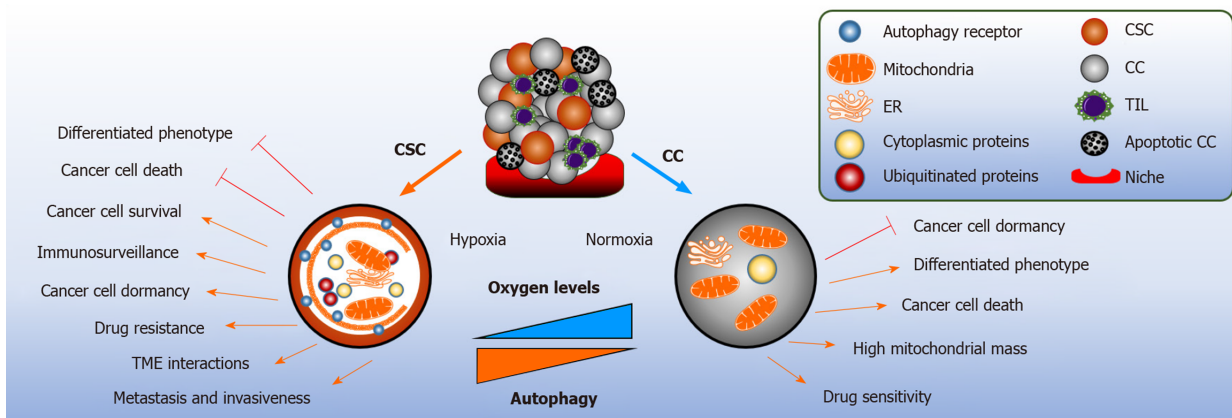


Figure 3 The divergent role of autophagy in cancer stem cells and cancer cells. Cancer stem cells (CSCs) drive the initiation and progression of cancer in multiple tumors. CSCs are reliant on their niches to sustain their self-renewal capacity and plasticity. Hypoxia induced autophagy, provides metabolic plasticity to CSCs. The role of autophagy in hypoxia is to modulate the metabolic remodeling of cancer cells, in particular CSCs. Additionally, autophagy and hypoxia have been implicated in immunosurveillance of CSCs during glucose limitation by increasing the expression of programmed death ligand 1 which results to tumor-infiltrating lymphocytes exhaustion. In addition, autophagy supports tumor dormancy, metastasis and invasion resulting to the treatment of resistant CSCs. CSC: Cancer stem cell; CC: Cancer cell; ER: Endothelial reticulum; TIL: Tumor-infiltrating lymphocytes; TME: Tumor microenvironment.

bulk autophagy degradation, in comparison to selective autophagy, such as mitophagy^[2]; in such instances early stage autophagy inhibitors would be considered to be more beneficial. Early stage autophagy inhibitors could target the initiation of autophagy, for example PIK-III (Vps34 inhibitor)^[144], MRT68921, SBI-0206965 (ULK inhibitors)^[145,146] and SAR405 (PIK3C3/Vps34 inhibitor)^[147]. Interestingly, SAR405 and Everolimus (an autophagy inducers) demonstrate significant synergism in renal tumor cells by reducing cancer cell proliferation^[147]. Additionally, early stage autophagy inhibitors would be a strategic method to target tumors grown in oxygenated environments, as they use OXPHOS as an alternative source of metabolism.

Autophagy is an adaptive mechanism modulating the TME surrounding CSCs. Several studies defined CSCs inducing autophagy in the TME to support their stemness and cancer propagation by activating the autophagic machinery under nutrient depleted and hypoxic conditions, for example in breast cancer^[72-74]. By these actions, autophagy can initiate the development of an aggressive cancer phenotype and develop resistance to cell death. Further investigations are needed to explore the role of autophagy in these cells within the tumor niche in order to tackle the protective surroundings of the TME.

INTERACTION BETWEEN AUTOPHAGY AND IMMUNOTHERAPY

Oncolytic viruses (OVs) therapy is an emerging anti-cancer treatment capable of efficiently killing CSCs and cancer cells in several tumor types^[148]. The most commonly used OVs include adenoviruses, herpes simplex virus, measles virus, reovirus, Newcastle disease virus and adenovirus serotype 5^[149]. OVs retain the capability to infect, replicate and integrate into tumor cells and potentially in their immunosuppressed TME. Malignant cells overexpressing certain virus receptors, including coxsackie-adenovirus receptor^[150,151], CD155^[152], CD46^[153] and laminin^[154] are targeted by OVs. Several studies revealed that autophagy facilitates immunogenic cell death *via* stimulating the release of pathogen associated molecular pattern and damaged associated molecular pattern and initiating their responses in the TME^[155]. These responses activate the secretion of ATP from the tumor cells promoting the stimulation of antigen presenting cells, such as DCs to elicit antigens on major histocompatibility I and II molecules which stimulate T cells^[13,156,157]. Consequently, pro inflammatory cytokines, including type I interferons induce the stimulator of interferon genes signaling in DCs, further benefiting anti-tumoral T cell responses^[158]. In the context of autophagy, OVs employ strategic methods to survive and propagate within the cancer cells by perturbing the core autophagic machinery^[159,160].

Autophagy can either be promoted or inhibited during oncolytic adenovirus therapy^[161]. The expression of adenovirus oncoprotein triggers the upregulation of Atg1, Atg5 and LC3 proteins^[162]. Leukemic cells treated with oncolytic adenovirus

encoding *BECN1* (*SG511-BECN1*) significantly induced autophagic cell death *in vitro*. Similarly, primary blasts isolated from chronic myelocytic leukemia patients with Imatinib resistance and AML patients with relapse disease treated with *SC511-BECN1* showed an increase in *BECN1* expression and LC3B accumulation. This led to significantly reduction of colony formation in comparison to *SG511* control^[163]. Interestingly, combination treatment of *SG511-BECN1* and Doxorubicin is highly synergistic in chronic myelocytic leukemia cell lines leading to significant cancer cell death. Increased levels of *BECN1* and LC3B proteins were observed in comparison with normal mononuclear cells; suggesting the combination of *SG511-BECN1* and Doxorubicin elicits synergistic effects in an autophagy dependent manner^[164].

In liver CSCs, oncolytic virus expressing tumor suppressor gene, *TSLC1*, and specifically targeting Wnt signaling, promoted the generation of autophagosomes. This was confirmed by the upregulation of *BECN1* and accumulation of total LC3 and led to the reduction of p62 and Survivin. This resulted in cell death in an autophagy dependent manner. The inhibition of autophagy by CQ induced the accumulation of total LC3 and p62, this in turn promoted the survival of the liver CSCs. The hepatic xenograft models treated with this adenovirus induced apoptosis and inhibited tumor metastasis resulting in an improved survival outcome^[165]. It has been proposed autophagy activators, such as Rapamycin or Temozolomide synergistically sensitize tumor cells to adenovirus by stimulating autophagy, without modifying the viral replication; thus, inducing autophagy dependent cell death as an antitumor mechanism^[166]. In addition, the adenovirus E4 protein suppresses autophagy by activating mTOR signaling and inhibiting ULK1 activity^[161].

IMMUNE CHECKPOINT INHIBITORS IN MODULATION OF AUTOPHAGY

The clinical development of immune checkpoint inhibitors (ICIs) is an emerging treatment modality for the reversal of TILs dysregulation phenotype, thereby imposing antitumor responses. Different immune checkpoints, such as T lymphocyte antigen-4 (CTLA-4), PD-1 and PD-L1 could be clinically targeted using ICIs^[167].

It is reported that PD-L1 expression on melanoma and ovarian cancer cells elicits tumor growth mainly *via* Akt-mTOR regulated autophagy; this data corroborated with a comparative microarray analysis. Moreover, melanoma PD-L1^{High} expressing tumors demonstrated increased sensitivity to CQ; thus, limiting proliferation *in vitro* and *in vivo*^[168]. RNA sequencing data in PD-L1 positive glioma cells promoted cancer invasion in starvation induced autophagy, utilizing the Akt-F-Actin signaling^[169]. In gastric cancers the knockdown of *Atg5* and *Atg7* genes inhibited LC3B formation, leading to the upregulation of PD-L1 by the activation of NF-Kb pathway^[170]. These accumulating studies confirm intrinsic PD-L1 functions through the activation of Akt-mTOR pathway, however, the mechanisms by which PD-L1 transduces signals remains unknown. The identification of these targets may potentially lead to targeted combinational treatments using autophagic agents. Recently, it is reported that Sigma1 promotes the degradation of PD-L1 using selective autophagy and ablates the functional interaction of PD-1 and PD-L1 in co-cultures of T cells and tumor cells^[171]. In accordance, targeting cancer cells expressing CD274 with PD-L1/PD-1 inhibitors can stimulate autophagy and promote sensitization of cancer cells when combined with autophagy inhibitors^[172].

CTLA-4 inhibitor is an effective ICI in a subset of patients with metastatic melanoma. In a small cohort of melanoma patients, a subcluster of MAGE-A cancer germline antigens, were found to be overexpressed causing resistance to CTLA-4 inhibition, but not PD-1. Tissue microarray data revealed that the LC3B expression in MAGE-A⁺ tumors was significantly attenuated as compared to MAGE-A⁻ tumors. Moreover, immunohistochemistry data indicated MAGE-A and damaged associated molecular pattern protein high-mobility group box 1 (HMGB-1) were mutually expressed in the clinical samples. *In vitro* ubiquitination screening confirmed that autophagy was suppressed by the MAGE-TRIM28 ubiquitin ligase complex^[173]. HMGB-1 is a pro autophagic protein that directly interacts with *BECN1* by displacing BCL-2; thus, sustaining autophagy and promoting cellular survival^[174]. The secretion of HMGB-1 mediates the priming of immune adaptive response^[175]. To overcome CTLA-4 therapy resistance in melanoma patients, the induction of autophagy may potentially be relevant in enhancing the effect of CTLA-4 inhibitors; thus, minimizing tumor immune tolerance. Combining CTLA-4 inhibition with Rapamycin *in vivo* during CD8⁺ T cell priming, led to an increase of Ag-specific memory CD8⁺ T cells and enhanced their function, which in turn, resulted to tumor growth reduction, rapid bacterial clearance and mediated cytokine production^[176]. Taking these findings into

consideration, the induction of autophagy would reinstate the CTLA-4 expression and its suppressive functions, thereby, eliciting antitumoral activity.

CONCLUSION

New therapeutic concepts are needed to improve the prognosis of cancer patients. One possible starting point is the tumor-specific metabolism of cancer cells. Autophagy is a catabolic recycling process exciting different forms of cancer cells and CSCs. In general, CSC maintenance and the development of an aggressive cancer phenotype have strongly been correlated to autophagy. In cancer, the role of autophagy is context dependent as it demonstrates functions both as a tumor suppressor during tumor initiation and as a pro-survival mechanism during cancer propagation by facilitating CSCs and cancer cells adaptive responses during metabolic stresses and dormancy.

Targeting autophagy could potentially represent a promising therapeutic target for preventing the aggressive and resistance cancer phenotypes. There is convincing evidence that the inhibition of autophagy in cancer cells, and specifically in CSCs, augments cytotoxicity leading to antitumoral effects under certain conditions. Therefore, we can expect valuable knowledge regarding suitable autophagy-associated biomarkers in tumor cells and new therapeutic approaches that are specifically directed against autophagy-dependent pathways in cancer cells or CSCs. Additionally, it is increasing evident that autophagy is involved in the maintenance of immune cell homeostasis, activation and function in the TME. However, limited studies are available to interpret whether autophagy enhancement or inhibition may support the effects of immunotherapy. Several additional preclinical studies are necessary to identify them, specifically, in a context dependent manner. This would represent an important step in the direction of improved and individualized cancer therapy.

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