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Recent progress of autophagy signaling in tumor microenvironment and its targeting for possible cancer therapeutics

Subhadip Mukhopadhyay [a,1], Kewal Kumar Mahapatra [a], Prakash Priyadarshi Praharaj [a], Shankargouda Patil [b], Sujit Kumar Bhutia [a,*]

[a] Cancer and Cell Death Laboratory, Department of Life Science, National Institute of Technology Rourkela, Rourkela 769008, Odisha, India
[b] Department of Maxillofacial Surgery and Diagnostic Sciences, Division of Oral Pathology, College of Dentistry, Jazan University, Saudi Arabia

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ABSTRACT

Autophagy, a lysosomal catabolic process, involves degradation of cellular materials, protein aggregate, and dysfunctional organelles to maintain cellular homeostasis. Strikingly, autophagy exhibits a dual-sided role in cancer; on the one hand, it promotes clearance of transformed cells and inhibits tumorigenesis, while cytoprotective autophagy has a role in sustaining cancer. The autophagy signaling in the tumor microenvironment (TME) during cancer growth and therapy is not adequately understood. The review highlights the role of autophagy signaling pathways to support cancer growth and progression in adaptation to the oxidative and hypoxic context of TME. Furthermore, autophagy contributes to regulating the metabolic switch for generating sufficient levels of high-energy metabolites, including amino acids, ketones, glutamine, and free fatty acids for cancer cell survival. Interestingly, autophagy has a critical role in modulating the tumor-associated fibroblast resulting in different cytokines and paracrine signaling mediated angiogenesis and invasion of pre-metastatic niches to secondary tumor sites. Moreover, autophagy promotes immune evasion to inhibit antitumor immunity, and autophagy inhibitors enhance response to immunotherapy with infiltration of immune cells to the TME niche. Furthermore, autophagy in TME maintains and supports the survival of cancer stem cells resulting in chemoresistance and therapy recurrence. Presently, drug repurposing has enabled the use of lysosomal inhibitor-based antimalarial drugs like chloroquine and hydroxychloroquine as clinically available autophagy inhibitors in cancer therapy. We focus on the recent developments of multiple autophagy modulators from pre-clinical trials and the challenges in developing autophagy-based cancer therapy.

1. Introduction

Autophagy, a lysosome-based conserved catabolic process involving degradation of the cellular materials and protein aggregates to maintain homeostasis. During stress, long-lived proteins or damaged cellular components are sequestered within a double-membrane structure called autophagosome and fuses with the lysosome to produce autolysosome, which is involved in the *de novo* release of amino acids, fatty acids and substrates to fuel cellular metabolism. The term "autophagy" was first coined by Nobel Laureate Christian de Duve during Ciba Foundation Symposium on Lysosomes, in London on February 12–14, 1963 [1]. The 1990s saw a tremendous exploration in the field of autophagy and its direct involvement in different diseases to highlight its physiological impact. The process of autophagy is controlled by 41 ATG (AuTophaGy-related) proteins which are highly conserved from yeast to mammals. Talking about yeast, it has played a vital role as a model organism to understand the process of autophagy progression. In 2016, Yoshinori Ohsumi was awarded the Nobel prize in physiology or medicine for his contributions to elucidate and explain the basis of the genetic mechanism of autophagy. Besides the above-explained process of autophagy (which is part of macroautophagy), the other two less known categories of autophagy are microautophagy and chaperone-mediated autophagy. Microautophagy involves direct uptake of autophagic cargo by lysosomes through invagination of their limiting membrane, whereas in chaperone-mediated autophagy, the autophagic cargo is loaded onto the lysosome in a process involving chaperone Hsc70 and lysosomal

* Corresponding author at: Cancer and Cell Death Laboratory, Department of Life Science, National Institute of Technology Rourkela, Rourkela 769008, Odisha, India.
E-mail address: sujitb@nitrkl.ac.in (S.K. Bhutia).
[1] Present address: Laura and Isaac Perlmutter Cancer Center, Department of Radiation Oncology, NYU Medical School, New York, NY 10016, USA.

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membrane protein LAMP2A [2]. Autophagy has a Janus character in cancer. On the one hand, autophagy involves in the elimination of cells that are prone to undergo tumor development, but once it develops, bypassing all regulation, then autophagy helps in nurturing them. This makes the role of autophagy not limited to only protection to the host individual, but it diversifies into an undesired phenotype promoting cancer recurrence, development of stem-like lineage properties, promotes malignancy and invasiveness (as shown in Fig. 1).

Autophagy is context-dependent on the tumor microenvironment (TME) and differs from the early to late stage of tumorigenesis. The TME exploits autophagy to fuel the metabolic demands of cancer stem cells (CSCs), fibroblasts, immune cells, lipid bodies, neuronal supplies controlling communication, blood vessels essential for nutrition, and the presence of microbiota, is housed in an impenetrable niche (as shown in Fig. 1). The concept of TME dates to Steven Paget's (1889) soil, and seed theory of metastasis, where the relevance of TME (soil) to support the cancer cells (seed) was emphasized [3]. The seeding of the premetastatic niches to far distant organs leads to the development of polyclonal metastatic sub-clones. The importance of TME is also recognized as an essential feature among the evolving hallmarks of cancer [4]. It is becoming increasingly important that there is a shift from tumor-centric to TME centric treatment. For example, glioblastoma (GBM) represents one of the most aggressive forms of cancer with minimal therapeutic options. Pyonteck et al. showed that the tumor-associated macrophages (TAM) depend on the colony-stimulating factor (CSF) for survival, and administration of CSF-1R (an inhibitor of CSF1) has been reported in tumor repression in patient-derived glioma xenografts [5]. In addition, it showed that there is a decrease of M2-activated macrophage markers in impaired TAMs. Interestingly, the phosphatidylinositol 3-kinase (PI3K) pathway gets activated in these recurrent GBMs, which are driven by macrophage-derived insulin-like growth factor (IGF-1) and tumor cell IGF-1 receptor (IGF-1R) [6]. In this connection, the combination of CSF-1R with IGF1 and PI3K pathway inhibitors leads to enhanced survival advantage and a better therapeutic route for treating recurrent forms of GBM. Likely, the combination of CSF-1R with paclitaxel reduces metastatic breast cancer progression in the mouse model [7]. The TAMs inside a hypoxia tumor are responsible for the secretion of vascular endothelial growth factor A (VEGFA), which binds to the nearby cognate receptors in nearby blood vessels. VEGF produces a cascade of growth in neighboring endothelial cells that help in the vascularization of TME. Interestingly, cellular lactate leads to the secretion of C-C motif chemokine Ligand 5 (CCL5) in macrophages by stimulating Notch signaling [8]. Wang et al. deciphered that the epithelial to mesenchymal transition (EMT), a hallmark of metastasis in gemcitabine resistant pancreatic cancer cells, requires the Notch signaling activation [9]. Interestingly, $CD44^{+}$ $CD54^{+}$ gastric CSCs display high autophagic activity and

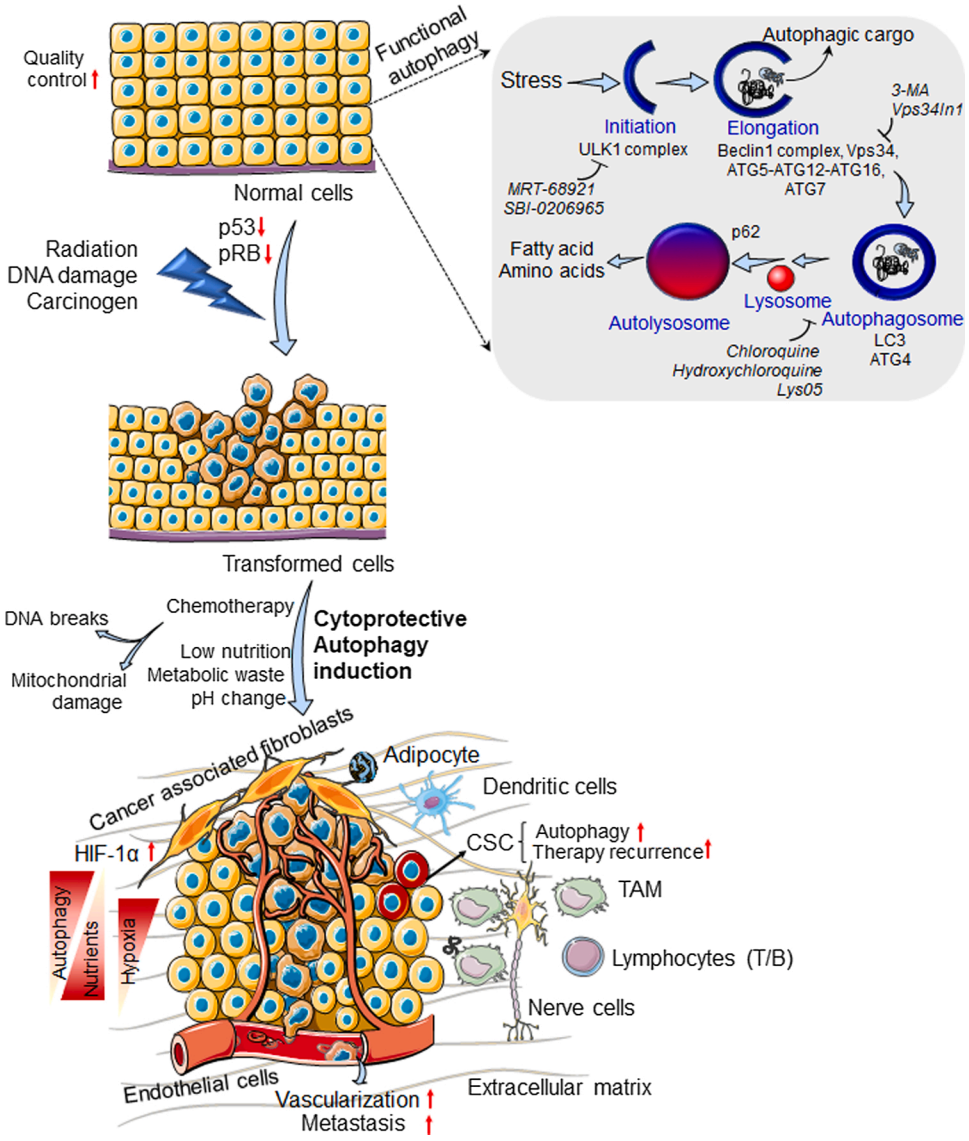

Fig. 1. Tumor microenvironment and autophagy in cancer: Normal cells undergoing functional autophagy involved in cellular quality control and senescence. These cells undergo cellular transformation through extrinsic (UV radiation, carcinogens etc) and intrinsic (genetic defects like loss of tumor suppressors viz. p53, pRb) routes. The stress inflicted in the tumor by chemotherapy, low nourishment, change in pH, accumulation of metabolic wastes triggers a tumor augmenting form of autophagy that supports the tumor ecosystem. The TME comprises tumor cells with sounding immune cells, fibroblast, adipocytes, neural connections, extracellular matrix. The core of the tumor lacks oxygen and nutrients leading to a high level of cytoprotective autophagy to give rise to CSCs population with high metastatic potential that also exhibit markers for therapeutic resistance. These cells continue to secrete growth factors, cytokines, and chemokines to alter the immune activation and tissue vascularization for cancer growth and progression. Up and down red arrows indicate upregulation and downregulation of respective parameters as indicated. In the inset (gray box), the brief schema shows the pathway of the autophagy progression along with their inhibitors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

combined treatment of chloroquine, and 5-fluorouracil (5-FU) promotes high Notch1 expression indicating the role of autophagy in regulating Notch-mediated chemoresistance [10].

This review has discussed evidence highlighting the role of autophagy signaling in modulating TME by reprogramming tumor metabolism, bypassing host immunity surveillance, and cancer stem cell development. We have discussed the present-day challenges of clinical trial strategies targeting autophagy to modulate TME and focus on future research goals for efficient cancer therapy.

2. Understanding the basics of autophagy signaling in cancer

During stress, cancer cells activate autophagy through a four-step catabolic process involving initiation, elongation, maturation, fusion with the lysosome, and degradation of the sequestered autophagic cargo. Stress, including high temperature, high interstitial fluid pressure, increase in the cancer cell population, hypoxia, accumulation of toxic waste products, radiation, and chemotherapy, evokes inactivation of the chief cellular signaling regulator mammalian target of rapamycin (mTOR) and activation of AMP-activated protein kinase (AMPK) to induce autophagy. It leads to the nucleation of the phagophore or a crescent-shaped double-membrane structure development. One of the prominent signaling complexes involved in this step is the Unc-51-like kinase (ULK) complex. ULK1, which is the yeast homolog of ATG1 comprised of major signaling player of this initiation complex; however, there is also the existence of other ULK isoforms like ULK2-4 and STK36. Although ULK2 can compensate for the role of ULK1 in some cases, however, very little is known about other isoforms. Although the exact mechanism of ULK1 in cancer is not yet fully understood, it has shown to be highly expressed in acute myeloid leukemia, colon, breast, and cervical cancers [11–14]. Deng et al. deciphered that ULK1 inhibition prevents the initiation step of autophagy and has a synergistic effect on programmed cell death protein-1 (PD-1) antibody blockage that resulted in augmentation of effector T-cell population and tumor regression in liver kinase B1 (LKB1)-mutant non-small cell lung cancers (NSCLC) [15]. The genetic method of ULK1 inhibition by a dominant-negative mutant dnULK1^{K46N} leads to the inhibition of metastatic neuroblastoma tumors by triggering apoptosis [16]. ^{V600E}BRAF-positive thyroid tumors are known to evoke cytoprotective autophagy for their survival through LKB1-AMPK-ULK1-pathway, and inhibition of autophagy along with ^{V600E}BRAF results in apoptosis induction [17]. Besides ULK1, the ULK complex comprises other proteins, namely ATG13, ATG101, focal adhesion kinase interacting protein of 200 kDa (FIP200), which remains inactive in optimal basal condition, but it gets activated upon inhibition of mTORC1 or when the AMPK pathway gets active. A knock-in of the FIP200-4A mutant mouse model showed the *in vivo* evidence for the essentiality of the ULK1/ATG13/FIP200/ATG101 complex to maintain tumor growth [18]. Interestingly, ULK1 stimulates autophagy by phosphorylating Beclin1 on Ser-14, which increases the activity of ATG14L-comprising vacuolar protein sorting 34 (VPS34) complexes [19]. Beclin1 inhibition has been proven to be a pivotal route to maintain chemoresistance towards paclitaxel treatment in BT-474 xenograft model and can be used as a prognostic marker for chemoresistant breast tumors [20]. Pre-autophagosomal protein ATG14 is recruited to the autophagosome biogenesis at the endoplasmic reticulum-mitochondria contact site by ER-resident SNARE protein syntaxin 17 [21]. ATG14 plays a crucial role in modulating oxidative stress, a key characteristic feature of TME. ATG14-induced lipophagy leads to reactive oxygen species (ROS) accumulation that controls ER-mediated mitochondrial apoptosis in HeLa cells [22]. Interestingly, the ER-Golgi site is also regarded as a site for LC3 lipidation and autophagosome biogenesis with the recruitment of ATG14 [23]. Wijshake et al. demonstrated that Beclin1 and UV radiation resistance-associated gene protein (UVRAG) but not ATG14 controlled the membrane localization of E-cadherin and metastasis [24]. The BATS domain of ATG14 targets the VPS34 complex to the nascent autophagic structures that senses the curved membranes enriched in phosphatidylinositol-3-phosphate (PI3P) [25,26]. The VPS34 inhibitor with anti–PD-1/programmed death-ligand 1 (PDL-1) therapy leads to antitumorigenic TME by producing pro-inflammatory factors including CCL5, chemokine (C-X-C motif) ligand 10 (CXCL10), and interferon (IFN)γ, which results in infiltration of natural killer (NK), T ($CD8^+$, $CD4^+$) cells to the tumor site [27]. The loading of WIPI2B on PI3P acts as a scaffold for generating the autophagosome, a double-ringed structure within which all the damaged cellular compartments are sequestered from the cytoplasm.

The curved isolation membrane continues to undergo elongation with the help of ATG7 and ATG10, which acts in the ubiquitin-like activating enzyme that gives rise to a complex, namely ATG5-ATG12-ATG16L1. Again, ATG7 and ATG3 complex is involved in the lipidation of LC3 (mammalian homolog of yeast Atg8) with the help of ATG4, thereby resulting in the formation of LC3-II (PE, Phosphatidylethanolamine conjugated) on the surface of autophagosome [28]. In this setting, LC3-associated phagocytosis (LAP) regulates TAM function to promote tumor burden, and it showed that loss of LAP in myeloid cells activates antitumor immunity [29]. This autophagosomal cargo machinery uses the kinesins and dynein motor machinery for its movement to reach different stressed portions of the cell, and finally, it fuses with the lysosome to give rise to the autolysosome. Autolysosome, in a layman's term, acts like a shredding machine for all the autophagic cargo load that is readily exported out as amino acids, free fatty acids, and other nutrients. These are made available to cancer cells for growth and progression, highlighting the efficacy of targeting autophagy and associated signaling for potential cancer therapeutics.

3. Identifying the role of autophagy in metabolic plasticity for rewiring cancer ecosystem

Altered metabolism is one of the hallmarks of cancer, and here, we have discussed the physiological implications of autophagy in regulating metabolic switch in cancer cells to survive in the TME (Fig. 2). Tumors prefer to use the anaerobic glycolysis and remain dependent on glucose, while the oxidative phosphorylation undergoing cancer relies on glucose, lipids, glutamine, and lactate. Glucose is one of the primary sources of carbon that fuels the metabolic demand in a proliferating cell. Glucose transporter 1 (GLUT1) is upregulated to maintain high glucose metabolism in breast cancer MCF-7 cells by increasing autophagy flux, and importantly, it also renders resistance to tamoxifen [30]. Autophagy-facilitated TBC1D5 retromer shuttling to the autophagosome controls plasma membrane positioning of GLUT1 for glucose uptake [31]. A combined regime of restricting the cells from extracellular sources like glucose deprivation (*in vitro*) or a 30 % calorie-restricted diet in comparison to control (*in vivo*) along inhibiting autophagy by deletion of Atg5 leads to highly efficient abrogation of Ras-driven tumors [32]. Interestingly, systemic loss of Atg7 through the whole body of mice system make them susceptible to hypoglycemia during fasting. At the same time, acute deletion of Atg7 in mice with pre-existing NSCLC leads to inhibition of tumor growth and generated benign oncocytomas [33].

Cancer cells exhibit the Warburg effect by virtue of which they are metabolically less reliant on mitochondria and utilize glycolysis mechanism even in the presence of oxygen. During glucose starvation, hexokinase-2 (HK2) is reported to transition glycolysis-dependent cells to autophagy-dependent pathways for metabolism by inhibiting mTORC1 [34]. HK2 plays an essential role in controlling autophagy, glucose-mediated lactate production, and EMT of tongue squamous cell carcinoma (TSCC) under hypoxia [35]. Knockdown of HK2 has reduced autophagic activity and blunted the invasiveness of TSCC. Ikeda et al. showed that loss of HK2 leads to apoptosis in the refractory form of multiple myeloma by abrogating glycolysis and autophagy [36]. Moreover, HK2 is also reported to induce cisplatin-resistant ovarian cancer cells by inducing extracellular signal-regulated kinase (ERK)-mediated autophagy in the TME [37]. Importantly, ubiquitination of HK2 by E3

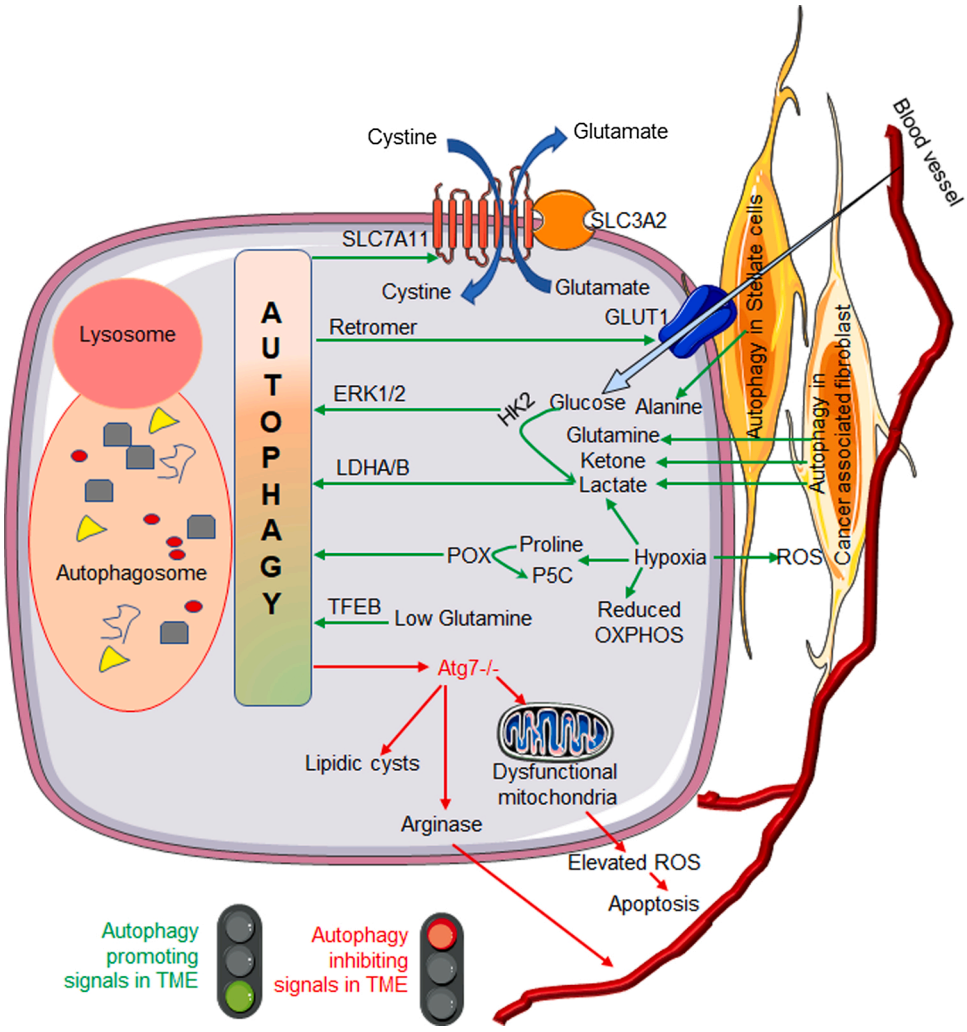

Fig. 2. Role of autophagy in regulating metabolic plasticity in tumor microenvironment: Autophagy maintains metabolites and biosynthetic intermediates for metabolic adaptation in cancer cells to sustain cancer growth and survival. Autophagy supports cellular glucose intake by upregulating cell surface expression of GLUT1 through retromer, while loss of autophagy leads to GLUT1 accumulation in the late endosome. Furthermore, HK2 plays an essential role in controlling glucose-mediated lactate production and autophagy through the ERK-dependent pathway and inhibiting mTORC1. LDH A, and B plays a critical in activating autophagy to protect the cells during oxygen-limited conditions. Similarly, glutamine deficiency triggers micropinocytosis-associated autophagy by activating TFEB to promote cancer cells survival. Cystine transporter SLC7A11 is dependent on autophagy-mediated plasma membrane localization for cystine import and balance of cellular antioxidants, while inhibition of autophagy leads to SLC7A11 inactivation. Autophagy-driven alanine secretion by the pancreatic stellate cells fuels the TCA cycle for the proliferation of cancer cells. Under hypoxia, tumor cells also trigger ROS-mediated autophagy in the neighboring CAFs to utilize high-energy metabolites including lactate, ketones, and glutamine for the TCA cycle to support growth and progression. During hypoxia, proline is metabolized into P5C by POX, resulting in ROS-mediated autophagy. Interestingly, loss of Atg7 results in impaired lipid metabolism and accumulation of lipidic cysts, and arginine depletion due to the presence of arginase in host circulation leading to growth suppression.

ligase tumor necrosis factor receptor associated factor 6 (TRAF6) at the Lys41 residue leads to its recognition by p62 as an autophagic cargo for degradation in liver cancer [38], indicating the complex role of autophagy to control glycolytic metabolism. Similarly, breast cancer cells execute autophagy through lactate dehydrogenase (LDH) A, leading to tamoxifen resistance with high expression of glycolytic proteins in TME [39]. Brisson et al. showed that lactate utilization occurs in SiHa cells through LDHB, which plays a critical role in autophagosome vesicle maturation along with lysosomal activity [40].

In low glucose conditions, glioblastoma cells depends on autophagic degradation of triglycerides, while inhibition of autophagy augments triglyceride reserves and leads to death [41]. In similar lines, Bosc et al. reported that inhibition of autophagy in acute myeloid leukemia (AML) leads to accumulation of lipid reserve and reduction of oxidative phosphorylation that inhibits leukemia proliferation along with disruption of the endoplasmic reticulum-mitochondria contact sites [42]. Intriguingly, endocrine therapy-resistant breast cancer cells can withstand long stretches of glucose starvation and utilize glutamine for survival by overexpressing the transcription factor c-MYC, possibly through autophagy [43]. Interestingly, starvation of glutamine but not lack of glucose is reported to trigger micropinocytosis-associated autophagy-mediated transcription factor EB (TFEB) induction in pancreatic ductal adenocarcinoma (PDAC) [44], indicating blocking autophagy and glutamine metabolism may be a crucial therapeutic strategy for pancreatic cancers. In this regard, Jeong et al. showed that mitochondrial glutamine anaplerosis inhibits autophagy in 8988 T pancreatic cancer cells to induce apoptosis [45]. Starving Atg7 deficient Kras-driven lung cancer cells are rescued by glutamine or glutamate, indicating the role of autophagy in nourishing tricarboxylic acid (TCA) cycle metabolites and nucleotide synthesis fuel [46]. Moreover, Atg7 deficient cancer cells display impaired respiration, struggled to cope with starvation, and depend on autophagy to sustain BrafV600E tumor growth and mitochondrial glutamine metabolism [47]. Likewise, glutamine insufficiency and suppression of mTOR induce autophagy in hepatocarcinoma cells by stabilizing AKT [48]. But autophagy-deficient colon cancer cells survive through upregulation of amino acids during glutamine deficiency, as evidenced by mTORC1 reactivation during this situation [49]. However, it is documented that glutaminolysis-mediated mTORC1 activation abrogates autophagy and results in the apoptotic killing of U2OS cells growing in limited nutrient conditions [50]. In this scenario, rapamycin treatment triggers protective autophagy by inhibiting mTORC1. Halama et al. reported that inhibition of glutaminolysis in breast cancer cells could be compensated by enhancing lipid catabolism and autophagy [51]. On the other hand, NSCLC patients expressing a mutant form of epidermal growth factor receptor (EGFR) exhibit a high level of glycolysis via c-Jun N-terminal kinase (JNK)-induced autophagy, so inhibition of autophagy can be a potential therapeutic route in lung adenocarcinoma [52].

Besides glutamine, other amino acids also significantly shape the ecosystem of TME through autophagy. Poillet-Perez reported that liver-specific Atg7 loss leads to arginase production that reduces circulating serum arginine and suppresses tumor growth [53]. Autophagy-driven secretion of alanine by the pancreatic stellate cells plays a crucial role in the growth and proliferation of pancreatic cancer [54]. It is shown

that the inhibition of autophagy also leads to the depletion of the cysteine pool in PDAC by impairing cystine transporter SLC7A11 [55]. Autophagy maintains the transport and translocation of SLC7A11 based on cysteine bioavailability. Tumor-derived cell lines bearing the loss of Atg7 and p53 have abrogation of survival and formed lipidic cysts highlighting the dysfunctional lipid metabolism [56]. Moreover, this strategy of autophagy impairment in the depletion of lipid reserve impedes LKB1-deficient Kras-driven lung tumorigenesis [57]. Fascinatingly, Su et al. demonstrated that autophagy inhibition in pancreatic cancer undergoes a metabolic switch to macropinocytosis through activation of transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) by autophagy cargo adaptor p62 [58].

4. Pro-survival autophagy signaling in hypoxic tumor microenvironment

Besides the role of autophagy in metabolic function, it plays an essential feature in adapting cancer cells in hypoxia (Fig. 3). Autophagy facilitates the survival of cancer cells in hypoxic TME through its main effector, hypoxia-inducible factor-1 (HIF-1). Tumor cells are reported to survive hypoxia by inducing Beclin1 mediated cytoprotective autophagy through BNIP3 and BNIP3L [59]. Furthermore, BNIP3L/NIX is highly expressed in patient-derived glioma tumor samples, which is required to maintain mitophagy during hypoxia through NFE2L2/NRF2 transactivation. Besides that, NIX overexpressing cells are prone to show glioma stem cell-like properties by triggering mTOR/AKT/HIF pathway [60]. Interestingly, glioblastoma cells are found to sustain the tumor growth even in regions of hypoxia by BNIP3 mediated autophagy through the HIF-1α-AMPK pathway [61]. Under hypoxic conditions, the damaged mitochondria are eliminated by triggering a crucial adaptor protein, FUNDC1, which plays a critical role in mitophagy by engaging with LC3 protein through the LC3 interacting region (LIR) of FUNDC1 via PINK1/PARKIN independent process [62]. In this case, autophagy inhibition leads to the transition of cytoprotective autophagy to apoptosis, preventing resistance to antiangiogenic therapy, including bevacizumab used in the clinic. Similarly, suppression of HIF-1α attenuates BNIP3 and Beclin1 mediated protective autophagy and triggers the effectiveness of gemcitabine-induced apoptosis in hypoxic bladder cancer cells [63]. On that note, $CD133^{+}$ pancreatic cancer stem-like cells also display increased HIF-1α mediated autophagy that regulates EMT under intermittent hypoxia [64].

HIF-1α triggers YTH domain family, member 1 (YTHDF1), to promote hypoxia-autophagy signaling by increasing ATG2A and ATG14 translation in hepatocellular carcinoma (HCC) [65]. Interestingly, ELP3-dependent PAK1 (K420) acetylation and PAK1-mediated ATG5 (T101) phosphorylation play a critical role in autophagosome maturation in hypoxic glioma cells [66]. The RNA splicing protein polypyrimidine tract-binding protein 3 (PTBP3) binds at 3′-UTR of ATG12, thereby increasing autophagic flux in PDAC during hypoxic condition confers resistance to chemotherapeutic agent gemcitabine resistance [67]. Furthermore, PRKCA/protein kinase C (PKC)α is identified as a kinase regulating hypoxia-mediated autophagy, which promotes tumor-initiating cell renewal that is reverted by loss of autophagy protein ATG5 and Beclin1 in colorectal cancer [68]. In this regard, the combinatorial effect of inhibition of autophagy with 3-Methyladenine (3-MA) and HIF-1α inhibitor (YC-1) could alter cisplatin-resistance in hypoxic conditions, thereby highlighting the translational significance in bladder cancer [69]. Similarly, 3-MA treatment inhibits hypoxia-promoted sorafenib resistance by reducing autophagy in HCC [70]. Conza et al. demonstrated that during hypoxia, mTOR downstream kinase and protein phosphatase 2 (PP2A) signaling routes govern the phosphorylation of prolyl hydroxylase domain-containing protein 2 (PHD2) to regulate HIF-1α mediated autophagy in the survival of colorectal cancer cells [71]. Again, yes-associated protein (YAP) promotes glioma cell growth by increasing high mobility group box 1 (HMGB1)-induced autophagy which highlights an exciting clinical

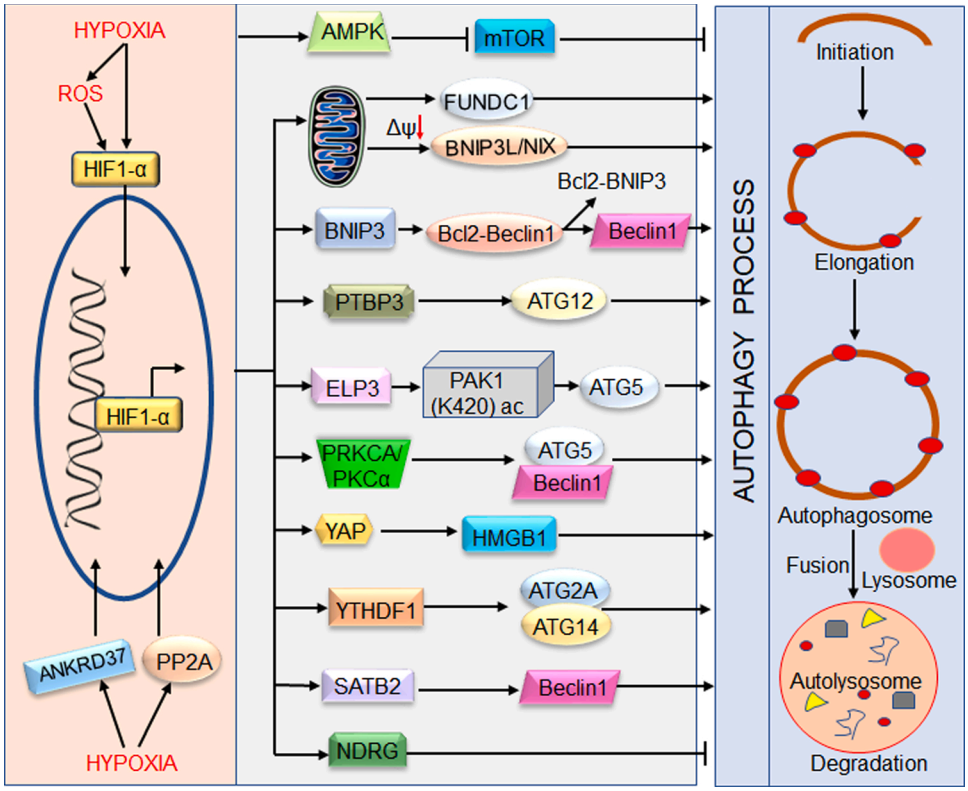

Fig. 3. Autophagic regulation of hypoxia and oxidative stress in tumor microenvironment: During hypoxia, tumor cells promote HIF-1α expression to induce Beclin1-mediated cytoprotective autophagy through BNIP3, an adaptive mechanism to stress. Furthermore, BNIP3L/NIX and FUNDC1 function as selective autophagy receptors to eliminate dysfunctional mitochondria and prevent ROS generation and cancer cell death. In addition, hypoxia in tumor cells promotes AMPK/mTOR-dependent autophagy for cell survival. Another primary mode of autophagy regulation during hypoxia occurs through HMGB1 signaling via YAP expression in cancer cells. Likewise, PAK1 acetylation and PTBP3 stimulate ATG5 and ATG12 mediated pro-survival autophagy, respectively. Moreover, PRKCA/PKCα is identified as a kinase regulating hypoxia-mediated autophagy through ATG5 and Beclin1 to protect tumor cells. Similarly, HIF-1α activates YTHDF1 to induce protective autophagy through ATG2A and ATG14 mediated pathways. The oncogene SATB2 increases hypoxia-mediated autophagy and stemness in cancer cells. On the other hand, NDRG1 inhibits autophagy at initiation and degradation step during hypoxia-mediated autophagy. ANKRD37 translocates to the nucleus and induces HIF-1α mediated autophagy in hypoxic cancers cells. Notably, mTOR downstream kinase and PP2A signaling routes regulate the phosphorylation of PHD2 to control HIF-1α mediated autophagy in the survival of cancer cells.

significance involving chemoradiation along with blocking autophagy in glioma patients [72]. In addition, autophagy in peritoneal mesothelial cells in gastric cancer under hypoxia promotes lysosomal-mediated degradation of sirtuin 1 (SIRT1), leading to HIF-1α acetylation and VEGF secretion to activate ERK/JNK pathway for peritoneal metastasis [73]. Deng et al. showed that ANKRD37 translocates to the nucleus and induces HIF-1α mediated autophagy in hypoxic colon cancers [74]. Meanwhile, inhibition of SATB2, an oncogene leads to a decrease in hypoxia-mediated autophagy and reduces the stemness potential in oral squamous cell carcinoma (OSCC) [75]. Another critical protein, N-myc downstream-regulated gene 1 (NDRG1), inhibits autophagy at initiation and degradation step during basal and hypoxia-mediated autophagy in pancreatic tumors [76].

Hypoxia-induced HIF-1α activates autophagy to maintain glycolytic phenotype for energy supply and cell survival [77]. Interestingly, hypoxia causes dysregulation of HK2 to promote autophagy, glucose consumption, lactate production, and EMT phenotypes in tongue cancer [35]. Similarly, HK2 augments anti-apoptotic functions through autophagy in HIF-1α/ HIF-2α dependent pathway in multiple myeloma in exposure to hypoxic stress, indicating the metabolic adaptation role of hypoxia through autophagy [36]. Similarly, under hypoxic conditions, proline oxidase (POX) plays a vital role in proline metabolism. Proline gets converted into pyrroline-5-carboxylate (P5C), which triggers ROS production mediated protective autophagy that ensures the survival of HT29 cells [78]. In this hypoxic situation, activation of POX is dependent on AMPK, but it operates in HIF-1α and HIF-2α independent way [78].

5. Autophagy, reactive oxygen species, and tumor microenvironment relationship

ROS comprise a family of highly reactive, short-lived molecules involving superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^•$). ROS is involved in the pathogenesis of cancer by oxidizing cellular lipids, damaging DNA integrity and proteins, making them susceptible to carcinogenesis and a more aggressive form of cancer. During tumorigenesis, the accumulation of dysfunctional organelles leads to ROS generation, which activates autophagy-mediated clearance of damaged cytoplasmic materials. Mice with Atg5 deletion in hepatocytes display oxidative stress and DNA damage with activation of p53, leading to hepatocarcinogenesis [79]. Recently, Kudo et al. reported that PKCλ/ι deficiency in hepatocytes triggers autophagy and oxidative phosphorylation (OXPHOS), leading to oxidative stress and activation of NRF2 [80]. Interestingly, PKCλ/ι is negatively correlated with HCC by preventing the activation of ROS and NRF2, establishing the complex role of autophagy, ROS, and carcinogenesis. Autophagy inhibits oxidative stress-dependent chronic inflammation and represents the tumor suppressor mechanism. For example, BRG1 controls the transcription of Atg16l1, Ambra1, Atg7, Wipi2 to induce autophagosome biogenesis to modulate colonic inflammation and colorectal cancer progression through oxidative stress-dependent autophagy pathway [81].

During tumor progression, autophagy has been established to be essential for cancer cells survival in exposure to different types of stress, including hypoxia and metabolic stress. In hypoxic TME, ROS promotes the expression of HIF1-α to induce mitochondrial autophagy through BNIP3 to eliminate dysfunctional mitochondria. BNIP3 disrupts the interaction of Beclin1 and Bcl2 to release the Beclin1 for autophagy induction as survival adaptive mechanisms to prevent ROS generation and cancer cell death. In another study, ROS in the bone marrow microenvironment activates the CXCR4-SDF1 signaling axis to promote autophagy for survival and maintenance of quiescent mantle cell lymphoma cells [82]. Moreover, cancer cells reprogram the metabolism through autophagy leading to recycling of high-energetic nutrients to sustain growth and progression. Furthermore, autophagy plays a pro-survival role by eliminating ROS accumulation due to increased OXPHOS during the consumption of TCA cycle intermediates to mitigate metabolic stress for cancer cell survival [83,84]. Metastasis is a multistep process that involves spreading cancer cells from the primary site to the distant secondary site. Autophagy-ROS crosstalk is involved in governing aggressiveness during malignancy. Loss of autophagy by Atg5 deletion or chloroquine treatment in pancreatic cancer leads to an increase in ROS accumulation involving DNA damage and metabolic switching from OXPHOS to glycolysis, indicating autophagy controls ROS and energy homeostasis resulting in malignant growth [83]. In another study, leucine-rich PPR-motif-containing protein (LRPPRC) stimulates cell migration, invasion, and glycolysis through the ROS/HIF1-α dependent pathway by inhibiting autophagy in retinoblastoma, establishing the complex role of autophagy in the regulation of ROS and cancer growth [85]. The detachment of epithelial cells from the extracellular matrix (ECM) induces anoikis with a decrease in ATP production and an increase in ROS production. It was showed that the ECM-detached cells exert PERK-ATF4-CHOP pathway leading to autophagy induction mediated through ATG6 and ATG8, thereby regulating ATP level and delay anoikis by reducing ROS cells in human breast ductal carcinoma *in situ* lesions [86]. The autophagy in metastasis is tumor suppressive in some instances and tumor-promoting in another context [87], and the role of ROS in this connection needs to be identified (Fig. 3). Autophagy activates focal adhesion dismantling to increase migration and invasion, leading to metastasis to lungs and liver through autophagic degradation of paxillin in metastatic mammary tumor model [88]. On the other hand, autophagy deficiency results in NBR1 accumulation to promotes aggressive tumor cell expressing keratin14 leading to pro-metastatic differentiation during tumor progression [89].

Autophagy in the stromal compartment plays a critical role in promoting tumor growth and invasion. Autophagy in cancer-associated fibroblasts (CAFs), immune cells, endothelial and adipocyte cells in the TME sustains tumor cell growth through the supply of nutrients and other factors [90]. For example, cancer cells under stress conditions produce ROS which transfer to CAFs to activate autophagy. The release of high-energy metabolites, including lactate, ketones, glutamine, and free fatty acids from CAFs fuel OXPHOS and other anabolic processes in cancer cells to support cancer growth [91]. A study on the *Drosophila* model showed that autophagy inhibition in the tumor cells exhibits significantly less effective as compared to the non-tumor epithelial cells surrounding the tumor or in the entire animal in restricting tumor growth and invasiveness [92]. Moreover, the autophagy inhibition through Atg4B dominant-negative in a pancreatic cancer mouse model results in tumor regression through modulating tumor cells and other components of TME, including macrophages [93]. However, the role of ROS in autophagy modulation on the crosstalk of tumor and associated stromal cells to maintain tumor growth need to be identified.

6. Autophagy promotes immune evasion to inhibit antitumor immunity

Evading immune surveillance is one of the hallmarks of cancer. The tumor evasion mechanism includes T cell anergy and resistance to apoptosis, deficiency of major histocompatibility complex (MHC) class I proteins, expression of immunomodulatory molecules in tumor cells. Moreover, the immune-suppressive factors present in the TME control the function of myeloid-derived suppressor cells (MDSCs), regulatory T (Treg) cells, and TAMs to promote tumor growth and metastasis. The current evidence has established that autophagy plays a tumor-protective role in tumor escape mechanism (Fig. 4 and 5). Kim et al. demonstrated that autophagy activation by NANOG through transcriptional induction of LC3B leads to EGF secretion that promotes EGFR-AKT signaling axis to exhibit resistance to $CD8^+$ T cells killing for $NANOG^+$ tumor cells [94]. Interestingly, LC3B inhibition sensitizes $NANOG^+$ immune-refractory tumor cells to adoptive transfer of T-cell and immune checkpoint blockade (ICB), resulting in inhibition of tumor growth. In another contrasting study, autophagy deficiency inhibits

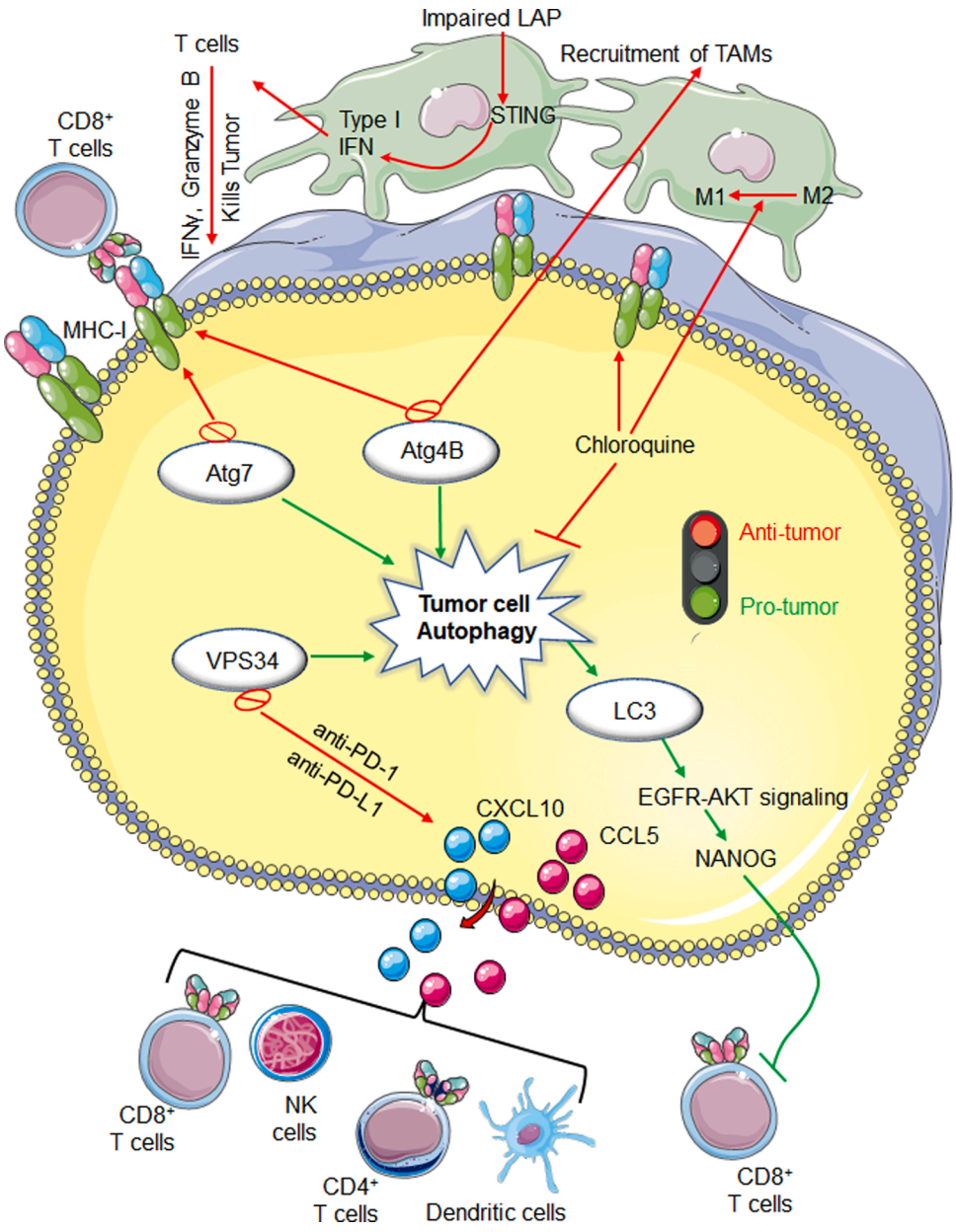

Fig. 4. Role of autophagy immune evasion to inhibit antitumor immunity: Upon loss of autophagy by knockdown of Atg7, or expression of dominant-negative Atg4B or chloroquine treatment, accumulation of MHC-I on the tumor cell surface to recognize and target by $CD8^+$ T cells. Similarly, inhibition of autophagy leads to infiltration of TAMs and the transition of macrophages from M2 to M1 with anti-tumorigenic activity. More importantly, impairment of LAP leads to STING mediated activation of T cells to produces IFNγ, granzyme B to kill the tumor cells. Inhibition of PIK3C3/VPS34 in combination with anti-PD-1 and PD-L1 increases tumor infiltration of CCL5 and CXCL10 along with NK and $CD8^+$, $CD4^+$ T cells. In the TME, LC3 protein is involved in the EGFR-AKT pathway that results in NANOG-mediated cytotoxic T cell inhibition.

$CD8^+$ T cell-mediated tumor killing by accumulating Tenascin-C in triple-negative breast cancer cells. Elevated Tenascin-C is correlated with poor prognosis of triple-negative breast cancer patients and exhibits an inverse relation with LC3B and $CD8^+$ T cells. Notably, targeting Tenascin-C for autophagic degradation through autophagy receptor p62 or inhibition of Tenascin-C in autophagy-impaired tumor cells improves the antitumor effect towards immune checkpoint inhibitors [95]. The immune checkpoint blocking therapy unresponsive colorectal and melanoma tumors were successfully treated by inhibiting Vps34 (using RNAi or SB02024, SAR405) with anti-PD-1/PD-L1 blockade. This results in an increase in pro-inflammatory cytokines and chemokines CCL5, CXCL10, and IFNγ along with NK and $CD8^+$, $CD4^+$ T-cells to enhance the efficacy of immunotherapy [96]. Recently Yamamoto et al. revealed that loss of autophagy by knockdown of Atg7 or expression of the dominant-negative form of Atg4B in tumor cells resulted in infiltration of $CD8^+$ T-cells within the pancreatic tumor [97]. Autophagy inhibition leads to an increase in surface expression of MHC-I molecules, thereby making them sensitive towards ICB therapy in pancreatic cancer. In addition, loss of Beclin1, Atg5, p62, or chloroquine treatment induces chemokine CCL5 expression in melanoma cells. More importantly, the autophagy defective TME leads to NK cells infiltration, leading to CCL5 transcription, and loss of CCL5 in the Beclin1 defective tumor cells reduces NK cells infiltration into the tumor site and inhibits tumor regression [98].

Autophagy in immune cells present in TME contributes as an immunosuppressive mechanism and controls antitumor immunity. It showed that autophagy stimulates in degradation of cytotoxic granules from NK and $CD8^+$ T cells to inhibit antitumor immune responses [99,100], and blocking hypoxia-induced autophagy restores tumor cell susceptibility to adoptive T-cell transfer and inhibition of PD-1. Moreover, lysosomal proteolytic activity is found to prevent the anticancer potential of $CD8^+$ T cells in melanoma cells [101]. Furthermore, $CD8^+$ T cells deficient in Atg5 have produced a higher amount of IFNγ and TNF-α and enhances tumor rejection in syngeneic tumor mouse models [102]. Autophagy activity in MDSCs induces immunosuppressive functions in melanoma patients [103]. Moreover, autophagy promotes MDSCs cell viability and survival through HMGB1 to drive immunosuppression and tumor progression [104]. Interestingly, the polyamine blocking therapy

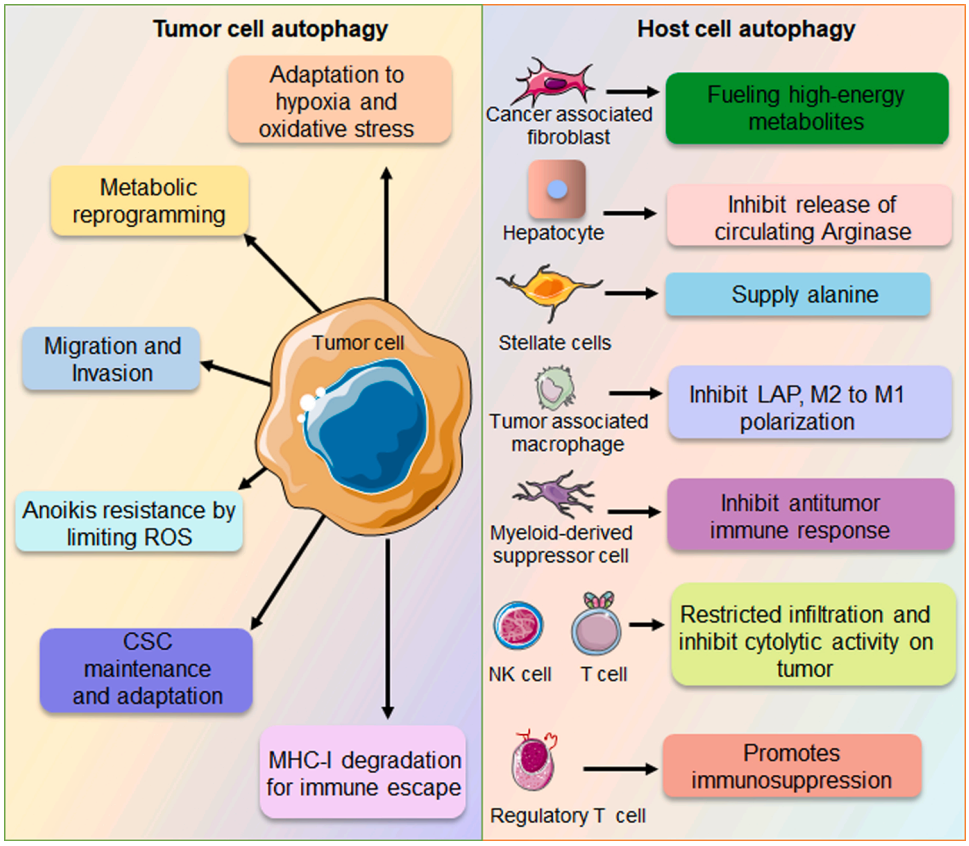

Fig. 5. A schematic representation of the role of tumor cell and host cell autophagy to determine the fate of a tumor cell in the tumor microenvironment.

inhibits cytoprotective autophagy in tumor-infiltrating MDSCs and macrophages and promotes the antitumor potential of PD-1 blockade [105]. The chloroquine leads to the transition of M2 to M1 phenotype of TAMs by activating inflammatory cytokines in lysosomal Ca^{2+} dependent signaling to promote antitumor $CD8^{+}$ T-cell immunity in B16 melanoma and H22 HCC tumor-bearing mice models [106]. The importance of non-conical autophagy, LAP is documented to contribute to M2 polarization in TME. Interestingly, LAP-deficient (Rubicon knockout) TAMs that have engulfed dying tumor cells augments pro-inflammatory response through increase expression of type-I IFN and IL-1β, leading to antitumor T cell responses to restrict tumor growth [29]. The combination of anticancer agents 5-FU and chloroquine augments the maturation of dendritic cells and leads to the stimulation of $CD8^{+}$ T-cell response towards HCT-116 colon cancer cells [107]. Interestingly, Treg cell-specific deficiency of Atg7 or Atg5 promotes impaired lineage stability and apoptosis in Treg cells leading to potent antitumor immune response [108].

7. Autophagy maintains and support survival of cancer stem cells in the tumor microenvironment

CSCs are a subset of malignant cells which drive cancer initiation, progression, and therapy resistance. The CSCs contribute and maintain tumor heterogeneity through activation of EMT, Juxtacrine, and inflammatory signaling in TME [109]. Here, we have discussed the role of autophagy in controlling growth, survival, and pluripotency in CSCs (Fig. 5). Autophagy in breast cancer positively regulates maintenance of CSCs, and Atg12 knockdown or chloroquine treatment showed a decrease in transforming growth factor beta 1 (TGFβ1)-induced accumulation of vimentin and increased expression of $CD24^{+}$ cells [110,111]. It showed that chronic myeloid leukemia (CML) exhibit ATG4B, ATG5, and Beclin1-mediated autophagy to maintain $CD34^{+}$ progenitor stem cell and suppression of Atg4B leads to inhibition of autophagy, which dampens the viability of CML $CD34^{+}$ stem cells and makes them sensitive to imatinib mesylate treatment [112]. Interestingly, Sharif et al. showed that disturbance of basal autophagy by using rapamycin or knockout of Atg7 decreases pluripotency in human and murine CSCs resulting in differentiation and/or senescence in the NAD^{+} biosynthesis pathway indicating pluripotency in CSCs involves autophagy homeostasis [113]. Importantly, autophagy maintains $ALDH^{+}$ and $CD29hiCD61^{+}$ breast cancer stem-like cells through EGFR/STAT3 and TGFβ/Smad signaling, respectively, in MMTV-PyMT transgenic mice [114]. In this setting, knockdown of Atg7 or Beclin1 decreases mammosphere-forming activity, $CD44^{+}/CD24^{low/-}$ cells, and interleukin (IL)-6 secretion, indicating autophagy promotes CSCs maintenance through IL-6 in breast cancer [115]. Autophagy activation by IL-17B/IL-17RB signaling controls CSC homeostasis. Interestingly, IL-17B recruits TRAF6 in the cytoplasm to trigger K63-mediated Beclin1 ubiquitination for autophagosome formation to drive self-renewal and sphere-forming potential in gastric cancer [116]. Similarly, IGF-2/insulin receptor (IR)-A signal controls autophagy to regulate pluripotency and stemness. Interestingly, IGF-2 loss of imprinting showed higher CD133 expression and sphere-forming potential by activating autophagy in CSCs in colorectal cancer [117]. Furthermore, autophagy promotes self-renewal activity in lung CSCs by degrading the ubiquitinated p53. Moreover, autophagic degradation of p53 enhances the expression of Zeb1 to regulates stemness, indicating the implication of the autophagy-p53-Zeb1 axis in controlling CSCs for self-renewal [118].

In hypoxic TME, autophagy promotes survival and metastatic ability of $CD133^{+}$ liver and pancreatic CSCs [119,120]. In this connection, hypoxic tumors induce calpain-6 expression through the stem cell transcription network, which associates OCT4, NANOG, and SOX2, and is involved with stem cell phenotypes in sarcoma. Intriguingly, calpain-6 knockdown promotes cellular senescence entry and inhibits autophagic flux suggesting calpain-6 regulates sarcoma stem cell fate in hypoxic

environments by controlling the balance between autophagy and senescence [121]. Likely, autophagy by melanoma differentiation associated gene-9 (MDA-9) promotes resistance to anoikis in glioma stem cells through phosphorylation of Bcl2 and EGFR signaling. Interestingly, inhibition of MDA-9 leads to glioma stem cells death in exposure to anoikis conditions [122]. Knockdown of Atg7 inhibits CD44 expression leading to a decrease in sphere formation, invasion, and lung metastasis in T24T bladder cancer cells. Mechanistically, ATG7 upregulates the deubiquitinase USP28, which removes the ubiquitin group from CD44 protein leading to stabilization of CD44 protein to control stem-like proprieties in bladder cancer [123]. Moreover, ATG7 is a target of miR-138-5p to maintain self-renewal and invasion in lung cancer-derived stem cells [124]. Interestingly, miR-138-5p mimic inhibits ATG7 dependent autophagy and stemness in lung cancer. Similarly, autophagy through the DRAM1-p62 axis maintains mitogen-activated protein kinase (MAPK) activation-mediated mesenchymal marker c-MET expression, ultimately governing migration/invasion in glioma CSCs [125].

CSCs display high autopahgic activity in maintaining therapy resistance in the tumor. For example, knockdown of ATG5 and Beclin1 or chloroquine treatment sensitizes the acquired radioresistant bladder cancer cell with high stem cell-like properties towards taxol [126]. It showed that autophagy promotes stemness in cisplatin-resistant oral squamous cell carcinoma, and inhibition of autophagy through knockdown of Atg14 results in reduced expression of CD44, ABCB1, and ADAM17 [127]. Furthermore, SOX2 promotes the expression of ABCC2, EMT, and exhibits chemoresistance through transactional activation of β-catenin in colorectal cancer. Interestingly, SOX2 increases expression of Beclin1 at the transcriptional level to trigger autophagy and drives chemoresistance *in vitro* and *in vivo*, indicating SOX2-β-catenin/Beclin1/autophagy signaling axis controls cancer growth and therapy resistance [128].

8. Present clinical status and challenges in autophagy targeted cancer therapy

The most recent progress of autophagy-targeted oncotherapy can be traced by understanding the clinical trials. Some of the critical ongoing clinical trials are listed in Table 1, while this section does a critical evaluation highlighting the success, failures of different trials. One such study named CHOICES (CHlorOquine and Imatinib Combination to Eliminate Stem cells) trial (NCT01227135) involved a phase II trial which is compared with co-treatment of imatinib mesylate (IM) and hydroxychloroquine, establishing a proof of concept of autophagy inhibitors use in leukemia treatment [129]. Moreover, a phase Ib/II report of bevacizumab with or without hydroxychloroquine showed that autophagy inhibition could revert chemoresistance in KRAS driven metastatic NSCLC [130]. Notably, hydroxychloroquine and temozolomide are documented to be safe, tolerable with antitumor benefits in phase I study in solid tumors as well as melanoma [131]. Again, another phase I study showed that hydroxychloroquine treatment with mTOR inhibitor temsirolimus leads to significant efficacy in tumor reduction [132]. However, Mehnert et al. showed in a phase I trial that the combination of hydroxychloroquine with AKT inhibitor MK-2206 in advanced solid tumors has limited antitumor activity [133]. In that line, the use of single-agent chloroquine in breast cancer trials has not shown any significant effect [134]. However, in combination with radiotherapy and temozolomide, chloroquine increases radiosensitization in EGFRvIII-expressing glioblastoma [135]. One interpretation could be that single use of autophagy inhibitors might not be the best treatment for cancer therapy, as evidenced by various instances previously reported [28]. Although, combinatorial use of chloroquine with sunitinib lead to inhibition of autophagy that improves clinical efficiency of sunitinib in treating pancreatic neuroendocrine tumor [136]. Inhibition of mitochondrial TCA cycle metabolism through CPI-613 (devimistat) along with chloroquine reduces the aggressiveness of soft tissue tumor growth and eradicated metastasis in clear cell carcinomas. Also, co-treatment of carfilzomib and chloroquine lead to robust induction of apoptosis *in vitro* and *in vivo* models of multiple myelomas [137]. Karasic et al. showed that a regimen involving hydroxychloroquine plus nab-Paclitaxel has an advantage in treating locally advanced PDAC where resection is needed [138]. A phase I/II trial of Everolimus and hydroxychloroquine treatment in patients with a previous history of renal carcinoma exhibits more than 40 % 6month progression-free survival [139]. However, hydroxychloroquine treatment in solid tumor patients in combinations with HDAC inhibitor vorinostat in phase I study displays the limited advantage of hydroxychloroquine addition [140].

Some of the significant challenges of the use of autophagy inhibitors during therapy are they have limited targeted delivery, which plummets the bioavailability of the drug. Moreover, continued use of drugs like chloroquine is reported to induce renal failure [141]. Also, chloroquine intake leads to cardiac damages and causes vagolytic effects [142]. The chloroquine and hydroxychloroquine also alter the voltage-gated Na^+, Ca^{2+}, K^+ ion channels, and pacemaker channels. However, hydroxychloroquine can offer higher bioavailability than chloroquine. In this setting, there is a need for the development of novel autophagy inhibitors for cancer treatment that could be specifically targeted and made available at ease to the tumor core, where most of the drugs fail to reach. One such example of advancement is the discovery of a Lys05 for anticancer treatment, which is found to be highly potent than hydroxychloroquine. Fascinatingly, in contrast to hydroxychloroquine, Lys05 is shown to exert single-agent antitumor activity without significant toxicity in mice [143]. Petherick et al. synthesized two drugs MRT67307 and MRT68921, which can specifically inhibit ULK1 and ULK2 to block autophagy [144]. Besides this, the combination of SBI-0206965 with mTOR inhibitors shows a potent tumor inhibition highlighting for clinical use [145]. However, one of the limitations of this molecule is that it could inhibit other kinases like FAK, FLT3, Src, and Jak3. Compared to SBI-0206965, the molecule ULK101 is reported to enhance potency and selectivity in KRAS-driven lung cancer, inhibiting autophagy nucleation [146]. Some of the most prominent VPS34 inhibitors that are showed to inhibit VPS34 complex-mediated autophagy progression are VPS34-IN1 [147] (Fig. 5).

Table 1
The ongoing cancer clinical trials using autophagy targeted therapy.

Clinical trial identifier	Treatment	Tumor type
NCT03037437	Sorafenib +Hydroxychloroquine (HCQ)	Hepatocellular carcinoma
NCT04214418	Cobimetinib+ HCQ + Atezolizumab	Gastrointestinal Cancer
NCT04386057	ERK inhibitor LY3214996 +HCQ	Pancreatic cancer
NCT03377179	Sphingosine kinase-2 (SK-2) inhibitor ABC294640+HCQ	Cholangiocarcinoma
NCT03979651	MEK inhibitor Trametinib +HCQ	Metastatic NRAS Melanoma
NCT04201457	Dabrafenib + Trametinib+HCQ	Glioma
NCT03598595	Gemcitabine + Docetaxel+HCQ	Osteosarcoma
NCT04163107	Carfilzomib +HCQ	Multiple Myeloma
NCT04524702	Paricalcitol +HCQ + Gemcitabine + Nab-Paclitaxel	Pancreatic cancer
NCT04132505	Binimetinib +HCQ	Pancreatic cancer

(source: https://www.clinicaltrials.gov/ct2/home).

9. Conclusion and future perspective

The heterocellular crosstalk through autophagy in TME often reprograms the cellular physiology for metabolic fitness and adaptation for tumor survival and growth. Autophagy in TME modulates metabolism, regulates oxidative stress and hypoxia, sustains cancer stem cells, and evades the host immune surveillance to support cancer growth (Fig. 5). Furthermore, it has become apparent that autophagy in host

tissues present in TME and the surrounding contribute to tumor growth. Although targeting autophagy to inhibit tumors is presently seen as a therapeutic measure, further research needs to be done to check their long-term effects on the host. Interestingly, the availability of autophagy inhibitors in cancer treatment is limited to chloroquine or hydroxychloroquine, and that too must be closely monitored for renal and hepatic problems if used for a prolonged period. It becomes extremely vital from the clinician's perspective to treat the autophagy inhibitor by analyzing the right stage, grade of ever-dynamic cancer.

There are many unanswered questions regarding the modulation of autophagy-based therapy for cancer and TME treatment, but it could expect new horizons of discovery. The focus and study could be on developing the new models to unravel the biological understanding of signaling that controls the interaction of cancer cells with the components of TME. In that regard, the reliance on the coculture model shows some important metabolic interactome. For example, Sousa et al. utilized coculture model to study the metabolic contribution of alanine from surrounding stellate cells in an autophagy-dependent process to fuel the progression of pancreatic cancer [54]. Moreover, the development of novel 3D coculture systems, including lab on a chip, can also contribute to a significant level to mimic the prognostically relevant niche of TME. Besides these cancer and host components in TME, it also becomes crucial to understand the modulation of the bacterial component of the tumor microbiome [148] on the autophagy level of cancer cells. While the development of technology, including DeepMACT, has facilitated the diagnosis of micrometastasis to study different sub-population of TME [149,150]. There is a need for further basic and translational research to elucidate these findings to address some of these unanswered questions for a better future of autophagy-targeted cancer therapy. The excitement over personalized tumor therapy harboring the TME status along with advancement in technology development to understand TME modulation in cancer progress will offer significant success.

Declaration of Competing Interest

The authors report no declarations of interest.

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