

Review

Dual Role of Lysosome in Cancer Development and Progression

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Abstract

Lysosomes are essential intracellular catabolic organelles that contain digestive enzymes involved in the degradation and recycle of damaged proteins, organelles, etc. Thus, they play an important role in various biological processes, including autophagy regulation, ion homeostasis, cell death, cell senescence. A myriad of studies has shown that the dysfunction of lysosome is implicated in human aging and various age-related diseases, including cancer. However, what is noteworthy is that the modulation of lysosome-based signaling and degradation has both the cancer-suppressive and cancer-promotive functions in diverse cancers depending on stage, biology, or tumor microenvironment. This dual role limits their application as targets in cancer therapy. In this review, we provide an overview of lysosome and autophagy-lysosomal pathway and outline their critical roles in many cellular processes, including cell death. We highlight the different functions of autophagy-lysosomal pathway in cancer development and progression, underscoring its potential as a target for effective cancer therapies.

Keywords: lysosome; autophagy; cancer; cell death; cell senescence

1. Introduction

Lysosomes, initially identified by Christian De Duve in the 1950s, are membrane-bound organelles containing more than 60 different types of hydrolytic enzymes that degrade various biological macromolecules [1–3]. Lysosomes ensure sustained autophagy function in cells. Functioning as digestive centers in eukaryotic cells, lysosomes play an important role in degrading unwanted substances, damaged organelles, microbes, and particles [4]. Beyond enzymatic functions, lysosomes also regulate multiple ions (e.g., H^+ , Ca^{2+} , Na^+ , Fe^{2+} , K^+ , and Zn^{2+}) through ion channels and transporters [4,5]. They rely on ions for their functions and reciprocally contribute to cellular ion homeostasis. Lysosomes utilize proton pumps, particularly vacuolar-type H^+ adenosine-triphosphatase (V-ATPase), to concentrate H^+ ions, thereby creating an acidic environment within the lysosomal lumen to enhance enzyme activity [6]. Moreover, lysosomes have the ability to store Ca^{2+} and regulate its concentration in the cytoplasm [7].

Traditionally viewed as cellular waste disposal units, lysosomes have experienced a significant paradigm shift in the last decades. They now function as dynamic and versatile organelles, serving as multifunctional signaling hubs that extend well beyond their conventional roles [8]. Notably, lysosomes actively participate in nutrient sensing, influencing cellular decisions through pathways like

mammalian target of rapamycin (mTOR) based on nutrient availability [9]. They also play central roles in immune cell signaling by degrading pathogens and presenting antigens, initiating immune responses [10–12]. Additionally, lysosomes are key players in cellular metabolism, breaking down macromolecules to provide essential building blocks for energy production [8,13,14]. This multifunctional role positions lysosomes as integral components in cellular homeostasis, adaptive responses, signaling cascades, etc. [15].

In recent years, research has revealed that aberrant lysosome function can lead to diseases including cancer and neurodegeneration disease [16–18]. This review delves into the connection between autophagy-lysosomal pathway and cancer, emphasizing how its role shifts throughout the stages of cancer development. During the initial stages, lysosomes act as tumor suppressors, maintaining cellular balance and eliminating mutated cells through processes like autophagy. As cancer progresses, lysosomes adapt to support increased metabolic activity, contributing to the sustained growth of cancer cells. The dual role of autophagy-lysosomal pathway, as both protectors and enablers, underscores their complex involvement in cancer development. Understanding these roles is crucial for developing innovative treatments and gaining insights into cancer development. In this review, we begin by summariz-



ing the roles of lysosomes in biological processes, particularly autophagy, to enhance understanding, and then discuss their functions and associated pathways in different stages of tumor development and progression, including both promoting and inhibiting effects on cancer.

2. Lysosome Biology in Autophagy

Autophagy is conserved degradation process that involves the delivery of cytoplasmic materials to lysosomes for breakdown and recycling [19,20]. This essential mechanism facilitates the turnover of proteins and organelles, contributing to cellular rejuvenation and survival [21,22]. More than 20 core autophagy-related (ATG) proteins regulate various phases of this process, including initiation, autophagosome nucleation, membrane deformation and autolysosome assembly [23–25]. Macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) are

three well-known types of autophagy that rely intricately on lysosomal function for the degradation of cellular constituents (Fig. 1) [26].

2.1 Macroautophagy

Macroautophagy, often known simply as “autophagy”, is considered the predominant type of autophagy, highly inducible in response to conditions like starvation and other stresses (Fig. 1a). The initiation of macroautophagy involves the formation of double membrane structure called the phagophore, which originates from the endoplasmic reticulum and may also derive from other sources, such as Golgi complex, mitochondria, and plasma membrane [27]. This process is regulated by the unc-51-like kinase (ULK) complex, consisting of ULK1, ATG13, focal adhesion kinase family interacting protein of 200 kDa (FIP200), and ATG101 [28], along with the

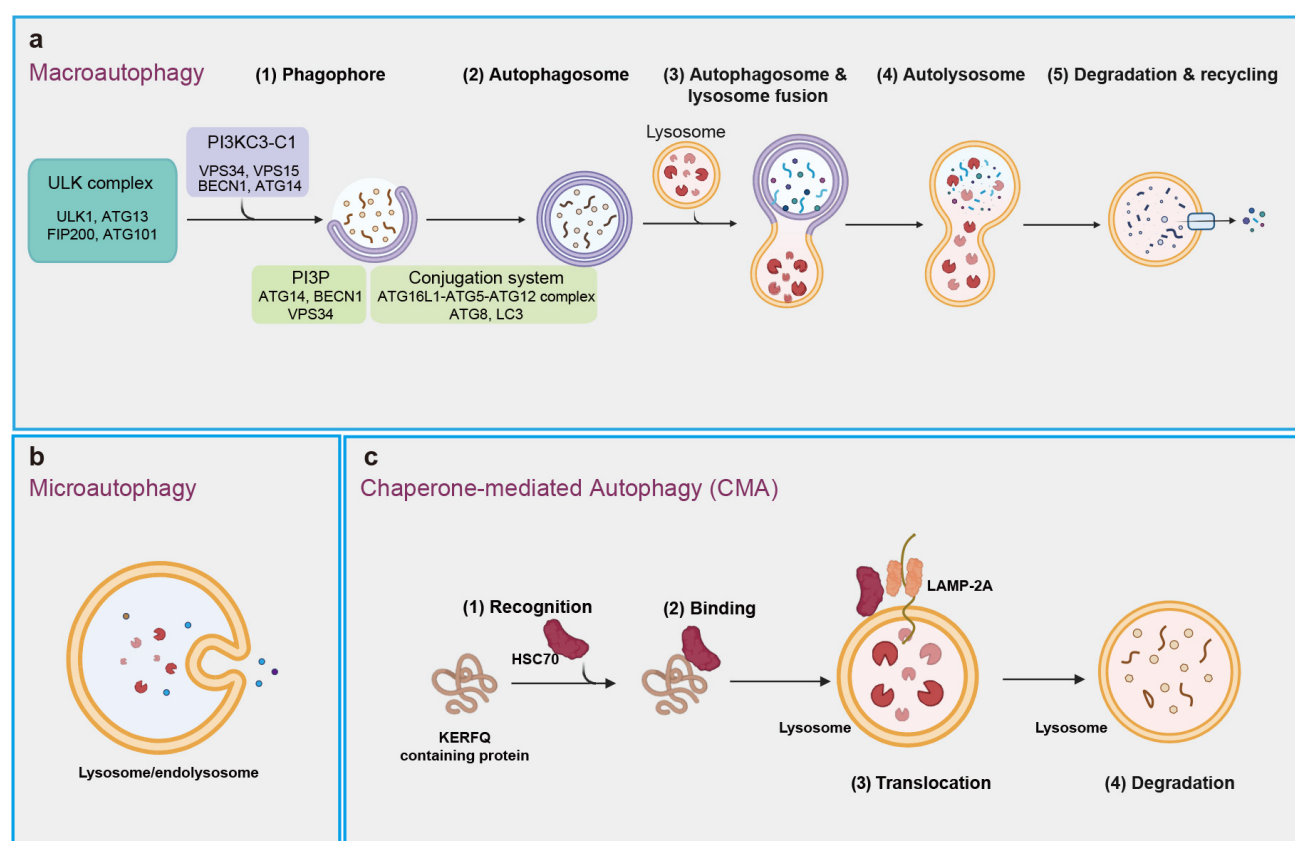


Fig. 1. Three main types of autophagy. (a) Macroautophagy includes a series of processes that begin with the formation of phagophore, which expands and seals to create autophagosome. This autophagosome then fuses with a lysosome, resulting in the formation of an autolysosome, where intracellular components are degraded and recycled. (b) Microautophagy involves the recognition of intracellular components by the lysosomal membrane, binding of these components through membrane invagination, and internalization into the lysosome for degradation. (c) Chaperone-mediated autophagy (CMA) is process in which chaperons (e.g., HSC70) recognize specific proteins with ‘Lys-Phe-Glu-Arg-Gln’ (KFERQ)-like motifs, transport them to lysosomal membrane, and facilitate their internalization through interaction with lysosomal receptor LAMP-2A for degradation. ULK, unc-51-like kinase; LAMP-2A, lysosome-associated membrane protein-2A; PI3KC3-C1, class III phosphatidylinositol 3-kinase complex I; VPS34, vacuolar protein sorting-associated protein 34; VPS15, vacuolar protein sorting-associated protein 15; BECN1, Beclin 1; ATG14, autophagy-related 14; PI3P, phosphoinositide phosphatidylinositol-3-phosphate; LC3, microtubule-associated protein 1A/1B-light chain 3.

class III phosphatidylinositol 3-kinase complex I (PI3KC3-C1), which includes vacuolar protein sorting-associated protein 34 (VPS34), VPS15, Beclin 1 (BECN1), and ATG14. PI3KC3-C1 binds to lipid membrane to generate phosphatidylinositol-3-phosphate (PI3P), a process facilitated by ATG14, BECN1 and VPS34 [29–31]. PI3P helps recruit the autophagy machinery proteins, including the ATG16L1-ATG5-ATG12 complex, which facilitate the lipid conjugation of the ATG8 family members, including the microtubule-associated protein 1A/1B-light chain 3 (LC3), crucial for cargo recruitment and autophagosome maturation. Subsequently, autophagosomes then fuse with lysosomes to form autolysosomes, where cellular materials are broken down and recycled [31–33]. Macroautophagy, categorized as non-selective or selective, involves the random engulfment of cytoplasmic components or the specific targeting of particular cargoes (e.g., protein complex) into autophagosomes for lysosomal degradation [34,35].

2.2 Microautophagy

Microautophagy refers to the process by which lysosomes or late endosomes (which fuse to form endolysosomes) directly engulf and degrade their internal contents [36] (Fig. 1b). This process occurs mainly through three mechanisms: lysosomal membrane protrusion, lysosomal membrane invagination, and late endosomal membrane invagination [37]. In addition, accumulating studies have shown microautophagy can selectively degrade specific proteins [36].

2.3 Chaperone-Mediated Autophagy

Chaperone-mediated autophagy (CMA) is a well-known type of selective autophagy, crucial for maintaining cellular homeostasis by degrading 30% of cytosolic proteins under conditions of prolonged nutrient deprivation [38] (Fig. 1c). CMA resembles biosynthetic pathways for proteins that to traverse membranes for entry into structures such as the endoplasmic reticulum or mitochondrion [38,39]. It can be upregulated in various conditions, including oxidative stress and genotoxic insults [40–42].

CMA primarily targets the soluble cytosolic proteins that contain a KFERQ-like consensus amino acid motif (Lys-Phe-Glu-Arg-Gln), for degradation in lysosome. Cytosolic chaperone heat-shock cognate protein of 70 kDa (hsc70) can recognize and bind the KFERQ-like motif of target proteins, which were then bind to lysosome-associated membrane protein-2A (LAMP-2A) on lysosomal membrane. Upon binding to LAMP-2A, the substrate protein is unfolded, translocated into the lysosome, and rapidly degraded [43–45].

3. The Lysosome as Cellular Signaling Hub

3.1 Lysosomal Nutrient Sensing and Metabolic Signaling

The lysosome plays a crucial role in sensing nutrients and regulating signaling pathways related to cellular

metabolism and growth. Notably, the mammalian target of rapamycin complex 1 (mTORC1) kinase complex, a key regulator of cell and organismal growth, can translocate to the lysosomal surface, where it inhibits autophagy [46]. mTORC1 is comprised of several core components, including mTOR, regulatory associated protein of mTOR (Raptor), and mammalian lethal with Sec13 protein 8 (mLST8) [47]. The lysosomal membrane is the primary site for the regulation of mTORC1 by amino acids. In the presence of amino acids, mTORC1 is driven to localize on lysosomes, while in their absence, it remains dispersed in the cytoplasm [48]. Amino acids rely on lysosome-bound heterodimers of Rag proteins to recruit mTORC1 to lysosomes, promoting its interaction with the Raptor subunit. Once localized to the lysosome, mTORC1 is activated, fostering the conversion of nutrients into essential macromolecules, fueling anabolic pathways [49] (Fig. 2).

In addition, lysosome is closely linked to the adenosine monophosphate-activated protein kinase (AMPK) network, which monitors cellular energy levels and triggers autophagy in response to energy depletion. While AMPK was previously thought to be localized in both the nucleus and cytoplasm, studies have revealed that it also localizes to the late endosome/lysosome, where it interacts with AXIN1 and orchestrates distinct lysosomal signaling pathways in response to glycolytic signals [50,51]. Aldolase senses fructose-1,6-bisphosphate (FBP) at the lysosome and transmits the glycolytic signal to regulate the formation of the AXIN-LKB1-Ragulator complex, activating AMPK at the lysosome [52]. Since AMPK and mTORC1 share the same lysosomal anchoring site, they may compete for activation at the lysosomal surface. While both can use the same downstream effectors, their effects are opposite: mTORC1 drives anabolic processes by suppressing autophagy, whereas AMPK promotes catabolism by stimulating autophagy [53,54].

3.2 Lysosomal Ion Homeostasis

Similar to the cytosol, lysosomes are membrane-bound organelles rich in various ions, including Ca^{2+} , Na^+ , K^+ , H^+ , Cl^- , Fe^{2+} , and Zn^{2+} , each of which exerts unique and indispensable physiological functions [5] (Fig. 2). Among these, Ca^{2+} is important for lysosomal trafficking and function [55,56]. As a universal signaling messenger, Ca^{2+} is involved in crucial for regulating various cellular processes, such as endocytic membrane trafficking, autophagy, and protein transport [57,58]. Lysosomal calcium efflux is mediated by three major types of channels: transient receptor potential mucolipin channel (TRPML), two-pore channel (TPC), and trimeric calcium two-transmembrane channels (P2X4) [59]. The TRPML family consists of three isoforms (TRPML1, 2, and 3), with TRPML1, primarily located in lysosomes, serving acts as an important positive regulator of autophagy by promoting the fusion of autophagosomes with lysosomes through the

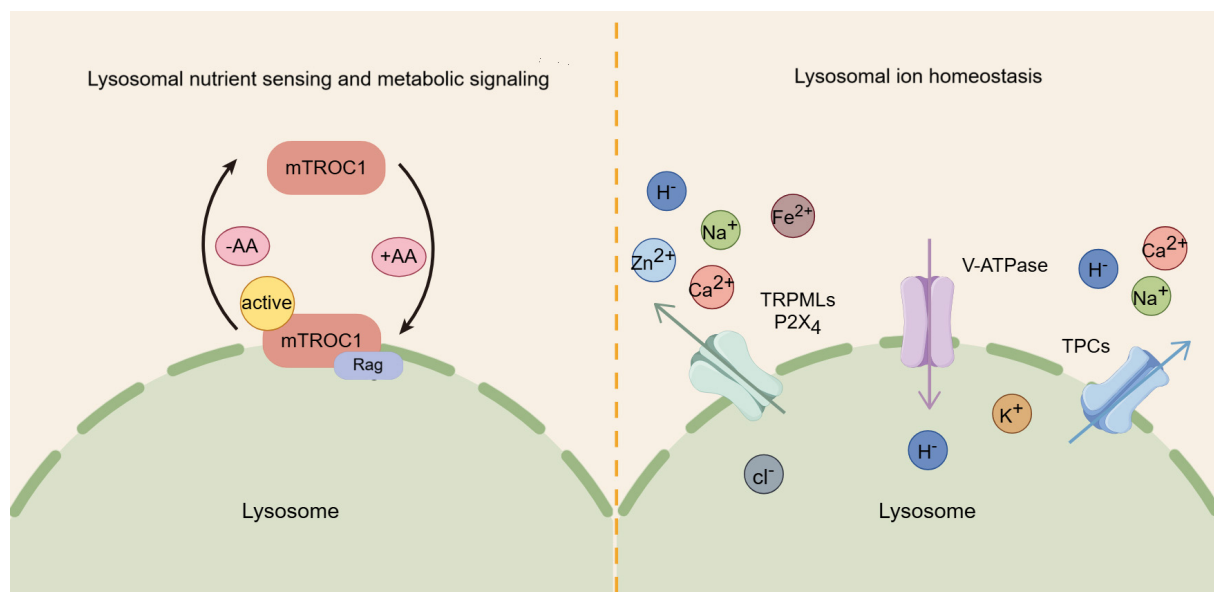


Fig. 2. The roles of lysosomes in nutrient sensing, metabolic signaling, and iron homeostasis. Amino acids (AA) activate mammalian target of rapamycin complex 1 (mTORC1) by promoting its binding to the lysosomal membrane, thereby regulating key metabolic signaling pathways. In addition, the lysosome serves as a crucial storage for various ions, and their transport, regulated by proteins like transient receptor potential mucolipin channels (TRPMLs) and two-pore channels (TPCs), is crucial for maintaining cellular ion homeostasis.

release of calcium ions [60,61]. TRPML2 and transient receptor potential mucolipin channel 3 (TRPML3) also participate in autophagy, with TRPML3 enhancing autophagy when overexpressed and inhibiting it when knocked down [62]. Additionally, TPCs, located on the lysosomal membrane, are likewise involved in calcium ion flux and the regulation of autophagy [63]. A study shows that TPC agonists can trigger Ca^{2+} release and inhibit the fusion of autophagosomes and lysosomes through increasing lysosomal pH [64]. Furthermore, P2X4 is also implicated in lysosomal function, becoming active during lysosomal exocytosis to facilitate processes such as surfactant secretion or phagocytosis [65,66].

In addition to Ca^{2+} , other ions also have significant biological functions. Monovalent cations such as Na^+ and K^+ constitute the primary positive charge within the lysosomal lumen [67]. Na^+ is necessary for the function of certain lysosomal transporters, including those from the SLC38 family [68,69], while K^+ regulates the lysosomal membrane potential and the homeostasis of lysosomal Ca^{2+} [69,70]. H^+ is essential for maintaining the activity of lysosomal digestive enzymes, most of which require an acidic environment to function optimally [5,6]. Cl^- acts as counterions to modulate the lysosomal membrane potential and, to a certain degree, aid in the acidification of the lysosomal lumen [71–73]. Fe^{2+} catalyzes the hydrolysis of H_2O_2 , producing reactive oxygen species and playing a crucial role in the lysosomal response to oxidative stress [74–76]. Zn^{2+} is a trace element that serves as an essential coenzyme for about 300 protein [77,78].

4. Complex Roles of Autophagy-Lysosome System in Cancer Development

The relationship between autophagy-lysosome system and cancer is complex and context-dependent. Autophagy serves as crucial homeostatic mechanism within cells contributing to cellular integrity, redox balance, and proteostasis by eliminating damaged organelles and proteins, thereby exerting a protective role against cancer [79,80] (Fig. 3). However, in the context of tumor progression, autophagy can also be hijacked by cancer cells to support their survival and growth [81]. Autophagy facilitates adaptation to the challenging microenvironments of tumors by supplying nutrients through the breakdown of cellular components, promoting cancer cell proliferation and metastasis (Fig. 3). In terms of therapeutics, ongoing research explores targeting autophagy as a strategy to either enhance or inhibit this process for more effective cancer treatments [82,83].

4.1 Autophagy's Role in Tumor Suppression

Autophagy is widely recognized for its crucial role in preventing tumor initiation in healthy tissues [81]. Before the onset of carcinogenesis, it acts as a key cytoprotective mechanism, mitigating metabolic stress and genome instability, thereby preventing tumor initiation [84]. Initial evidence supporting the tumor-suppressive role of autophagy emerged from a study on the autophagy-essential *BECN1* gene, responsible for encoding beclin-1 functioning in the formation of the phagophore [79]. Earlier research, such as a study reporting frequent allelic deletion

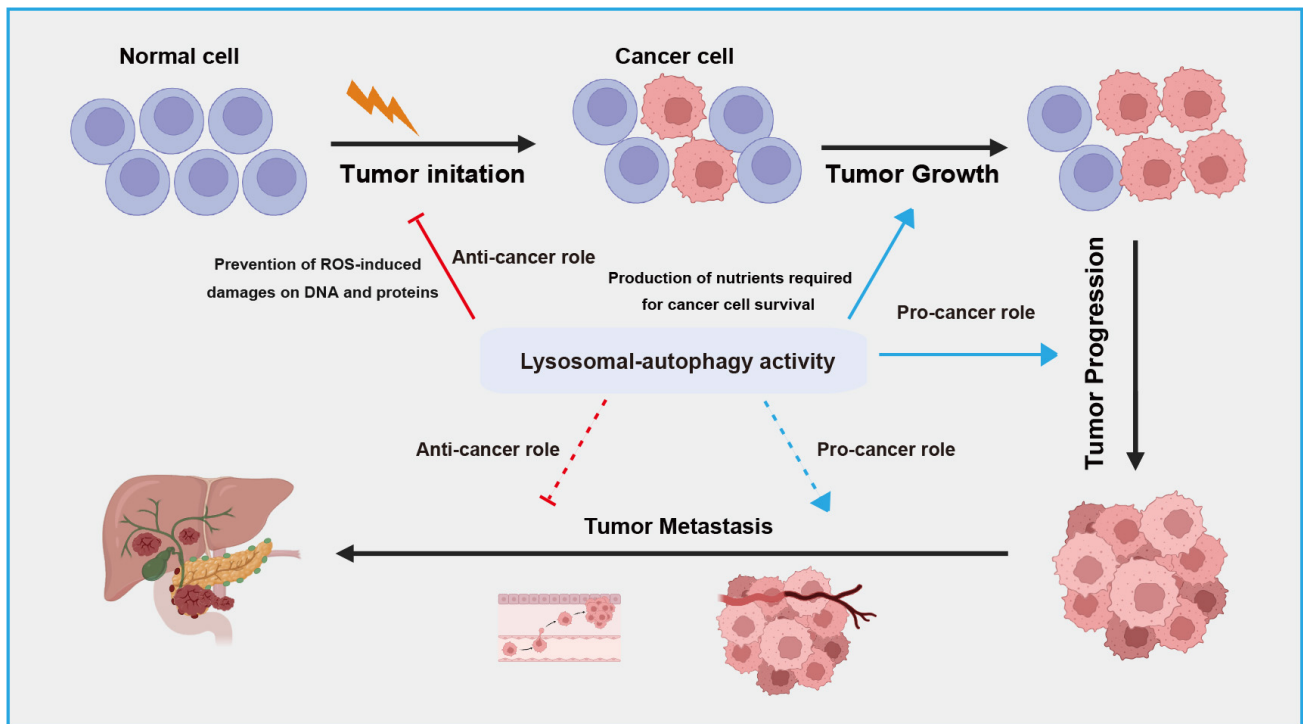


Fig. 3. A schematic overview of dual roles of lysosomal-autophagic pathway in different stage of tumor development. The lysosomal-autophagic pathway suppresses tumor initiation by degrading damaged proteins in normal cells but promotes tumor cell growth and progression by supplying nutrients. During tumor metastasis, this pathway exhibits both anti-tumor activity by limiting inflammatory responses and pro-tumor activity by promoting epithelial-mesenchymal transition. ROS, reactive oxygen species.

of *BECN1* in breast and ovarian cancers, suggesting that the loss of *BECN1* and potential defects in autophagy are implicated in tumorigenesis [85]. Additionally, both cancer cell lines and mouse models reveal that loss of *BECN1* leads to reduced autophagy and increased cell proliferation, further indicating the tumor suppressor role of the *BECN1* gene [79,86,87]. Moreover, a growing body of findings have shown that a large number of autophagy-related genes (e.g., *ATG5*, *ATG12*) are mutated or inactivated to evade the tumor suppressive effects of autophagy as tumor development progresses [88].

The production of reactive oxygen species (ROS), primarily comes from damaged mitochondria, significantly contributes to mutagenesis and, consequently, tumor initiation [89,90]. Research indicates that autophagy's elimination of damaged mitochondria leads to decreased ROS production, thereby limiting the tumor-promoting effects of ROS [91]. Moreover, studies have shown that inhibiting autophagy results in chronic oxidative stress and the accumulation of damaged mitochondria, promoting the initiation of tumor [92,93]. An increasing body of evidence suggests that autophagy plays a role in suppressing tumor initiation by degrading certain proteins known to drive tumorigenesis [84,94]. Among these, the oncogenic mutant p53 protein is particularly significant in this context, as it plays an important role in initiating tumor [95]. Autophagy, specifically through CMA, facilitates the degradation of mutant p53, au-

tophagy, particularly through CMA, thereby contributing to the suppression of tumor initiation [96].

4.2 Autophagy's Role in Tumor Progression

Once the primary tumor is formed, the role of autophagy in tumor progression becomes intricate and depends on the cellular context. The earliest evidence of autophagy's role in maintaining established tumors comes from the observation that certain tumor tissues exhibit high levels of LC3 puncta and lipidated LC3 (LC3-II), indicating the accumulation of autophagosomes in these tissues [97]. However, these static tissue-based readouts only show the levels of autophagosomes and are therefore largely unable to distinguish between the induction of autophagy or impairment of autophagosome turnover. Nevertheless, numerous preclinical studies have shown that autophagy facilitates the growth and metabolism of advanced tumors following the activation of various oncogenes and/or the inactivation of tumor suppressor genes [98,99].

In the advanced stages of tumors, autophagy promotes tumor development and increases tumor cell growth and metastasis [100,101]. Studies have shown that autophagy aids cancer cell survival at this stage by enhancing cellular tolerance to adverse conditions such as hypoxia and nutrient deprivation, thereby promoting tumor progression and recurrence. Autophagy is activated in the central regions of solid tumors where cells are often un-

der hypoxic conditions [102,103]. Additionally, inhibiting autophagy by deleting *Beclin 1* increases cell death. Specifically, the loss of *Beclin 1* leads to a loss of its regulatory function in autophagy, which in turn exacerbates apoptosis [104]. Tumor cells are characterized by a high metabolic rate, with greater demands for nutrients and energy than normal cells. Autophagy can recycle intracellular components to provide metabolic substrates, meeting the high metabolic and energy demands of proliferating tumors [105,106]. The degradation products of autophagy include nucleotides, amino acids, fatty acids, and sugars, which can be reused in intracellular synthesis and energy metabolism processes [107,108]. Additionally, autophagy helps cancer cells remove damaged or excess proteins and organelles, maintaining intracellular stability and thereby promoting tumor development [109,110]. In animal studies, metabolic stress has been observed in autophagy-deficient cells, leading to impaired cell survival [111]. Thus, autophagy promotes tumor cell survival by enhancing stress resistance and providing nutrients to meet the metabolic needs of tumors, whereas inhibiting autophagy or knocking down autophagy genes can lead to tumor cell death [80,112].

Some studies have shown high levels of autophagy in *RAS*-activated mutant cancer cells. Rat sarcoma (*RAS*) is a small GTPase involved in crucial signaling pathways for proliferation, survival, and metabolism [113]. *RAS* mutations are found in various cancers, including colorectal cancer, pancreatic cancer, and lung cancer, the development and progression of these cancers are closely related to the increased autophagy induced by *RAS* mutations. *RAS* mutations increase autophagy through various mechanisms, enhancing tumor growth, survival, and oncogenicity [114–117]. Additionally, inhibiting autophagy-related proteins increases the accumulation of damaged mitochondria and reduces cell growth. For example, p62 promotes the accumulation of damaged mitochondria through its Phox and Bem1 (PB1) oligomerization domain and plays a key role in mitophagy, where damaged mitochondria fuse with lysosomes to form mitophagolysosomes, thereby clearing damaged mitochondria [118]. Knocking down p62 prevents the effective clearance of damaged mitochondria, leading to their accumulation in cells, increasing oxidative stress levels, and ultimately affecting normal cell function and growth [119–122]. These results indicate that autophagy plays an important role in the survival of tumor cells dependent on *RAS* activation.

4.3 Autophagy's Role in Tumor Metastasis

Cancer metastasis is the leading cause of death in cancer patients, and autophagy plays a dual role in this process, both promoting and inhibiting metastasis [123]. In the early stages of cancer metastasis, autophagy acts to inhibit metastasis by limiting inflammatory responses, while also reducing the invasion and migration of cancer cells from primary sites (Fig. 3). A study has found that knock-

ing down autophagy-related genes such as *Beclin 1* inhibits the proliferation, migration, and invasion of breast cancer cells, leading to apoptosis [124]. Additionally, a study has shown that blocking the mTOR signaling pathway induces autophagic cell death and inhibits gastric cancer cell metastasis [125].

However, in the late stages of metastasis, autophagy promotes metastasis by enhancing the survival and colonization of cancer cells at secondary sites [126]. There is evidence that autophagy facilitates several biological pathways crucial for effective metastasis, including migration and invasion, regulation of epithelial-mesenchymal transition (EMT) [127–129], resistance to detachment-induced cell death (anoikis) [130,131], and adaptation to nutrient deprivation and hypoxia [105] (Fig. 3). Recent studies in various models suggest that autophagy may limit key rate-limiting steps in the metastatic cascade. For instance, D2.OR mammary cancer cells transplanted into syngeneic hosts display dormancy and do not develop into active metastases [132]. However, knocking down *Atg3* in these cells disrupts their dormancy, resulting in the emergence of proliferative metastatic cells with enhanced cancer stem cell-like traits. This suggests that inhibiting autophagy generates invasive subpopulations *in vivo* [133]. Further experiments demonstrated that conditionally deleting *Atg5* or *Atg12* genes in tumor cells that had already spread to the lungs resulted in a highly proliferative subpopulation with enhanced metastatic growth [134]. Comparable results were seen in experimental metastasis models using 4T1 mammary cancer cells with *Atg12* knockdown. In contrast, the genetic removal of *Rubcn*, a well-known inhibitor of autophagy, was effective in reducing extensive metastatic growth [134]. Overall, these studies indicate that autophagy acts as a stage-specific suppressor of metastatic colonization.

Additionally, p62 (encoded by *SQSTM1*) is a selective substrate for autophagy and is believed to play a role in promoting tumor initiation. Studies have demonstrated that p62 prevents the degradation of TWIST1, a key transcription factor that regulates EMT [98,135]. In mouse mammary cancer models, researchers observed that impaired autophagy causes an accumulation of NBR1, leads to the formation of aggressive tumor cell subpopulations exhibiting pro-metastatic basal differentiation traits. Functional studies have also demonstrated that increased NBR1 levels promote pulmonary metastatic colonization and the acquisition of these basal differentiation features [134]. These studies imply that the accumulation of p62 and NBR1 in the absence of proper autophagy is a significant factor in driving the metastatic phenotype.

5. Lysosomes and Tumor Cell Death Pathways

Persistent lysosomal damage significantly impacts cell fate. Research suggest that lysosomes mediate pro-

grammed cell death (PCD) through various mechanisms, including apoptosis, necroptosis, pyroptosis, and ferroptosis [16,136]. The degree of lysosomal damage may play a role in regulating the type of cell death: moderate lysosomal damage tends to induce apoptosis, whereas severe damage can result in irreversible necrosis.

5.1 Apoptosis

Apoptosis, a well-known type of PCD, is marked by caspase activation, chromatin condensation, nuclear fragmentation, and the formation of apoptotic bodies [137]. Research indicates that lysosomal membrane permeabilization (LMP) leads to the release of lysosomal intraluminal cathepsins into the cytoplasm, initiating apoptotic cell death through both caspase-dependent and -independent pathways, with or without involvement of mitochondria [138]. In addition, although lysosomes are not the primary storage sites for Ca^{2+} , a study has shown that the release of Ca^{2+} from lysosomes into the cytoplasm is critical for phosphatidylserine (PS) exposure and contributes to apoptosis [139]. LMP has also shown to cause a sustained accumulation of cytosolic calcium, which can precede apoptosis in cancer cells [140].

5.2 Necroptosis

Necrosis was traditionally viewed as a non-programmed form of cell death. However, research has shown that necrosis can be regulated by certain molecular mechanisms, a process known as necroptosis [141]. A well-studied mechanism by which lysosomes contribute to necroptosis also involves the release of cathepsins B and D into cytoplasm in response to LMP [142]. In addition, studies have also shown that increasing destabilization of lysosomal membranes can sensitize tumor cells to drugs [143], with the induction of LMP promoting necroptosis in cancer cells [144]. These findings suggest the potential of this pathway as a therapeutic strategy for targeting and eliminating tumor cells.

5.3 Pyroptosis

Pyroptosis is an inflammatory form of PCD characterized by the formation of pores in the plasma membrane, leading to membrane rupture and the release of inflammatory factors (e.g., interleukin 1β (IL- 1β) and IL-8). This process is primarily regulated by gasdermin (GSDM) family proteins like GSDMD and GSDME, which are cleaved into their active form by caspases such as caspase 1, caspase 3 and caspase 4/5/11, releasing their pore-forming N-terminal fragments [145]. GSDM-mediated pyroptosis has been observed in various cancer cells [146]. Furthermore, studies have suggested a significant link between lysosomes and pyroptosis [147]. During pyroptosis, lysosome exocytosis has been observed [147]. Additionally, the permeabilization of lysosomes leads to the release of cathepsin B, which can activate caspase-1, a crucial regulator of pyrop-

toxis, indicating the roles of lysosomes in the regulation of pyroptosis [148].

5.4 Ferroptosis

Ferroptosis is a regulated form of cell death characterized by the excessive accumulation of iron-dependent lipid hydroperoxides within cells, typically associated with the accumulation of intracellular iron [149]. The Fe^{2+} produced by the Fenton reaction or the inactivation of glutathione peroxidase 4 (GPX4), leads to the accumulation of lipid hydroperoxides that always triggers ferroptosis [150]. Lysosomes, being the center of iron metabolism, plays an important role in regulating ferroptosis [151,152]. A recent study has shown that inhibiting the lysosomal TRPML1 channel, a crucial protein involved in lysosomal iron homeostasis, including the release of Fe^{2+} from lysosome to the cytosol, can induce ferroptosis and eliminate breast cancer stem cells [153]. In addition, lysosomes regulate ferroptosis through autophagy-mediated degradation of proteins, such as GPX4, that are involved in the ferroptosis process in various cells, including cancer cell [154,155]. Furthermore, lysosomal cystine has been proved to influence ferroptosis in cancer cell via the aryl hydrocarbon receptor (AhR)-activating transcription factor 4 (ATF4) signaling pathway [156], indicating the multiple mechanisms by which lysosomes modulate tumor cell ferroptosis.

6. Lysosomes Link Cell Senescence and Tumor Progression

Alterations in lysosomal function, such as increased lysosomal number, size, and enzymatic activity, are prominent characteristics of senescent cells [157]. While cellular senescence was initially recognized as a tumor-suppressive mechanism that prevents the proliferation of abnormal cells and inhibits malignancy progression, accumulating evidence suggests that it also plays a crucial role in creating a pro-tumorigenic environment within local tissues. This environment is characterized by chronic inflammation and altered immune responses, ultimately promoting tumorigenesis [158]. Altered lysosomal function in senescent cells significantly contributes to this process. A key mechanism involves the secretion of various factors, including inflammatory cytokines, chemokines, and growth factors, collectively known as senescence-associated secretory phenotypes (SASP). These factors are released by senescent cells thorough lysosomal exocytosis [159], further influencing the tumor environment and facilitating tumor development. For instance, a study has shown that senescent cells secrete chemokines like interferon γ -induced protein 10 (IP-10) and regulated upon activation normal T cell expressed and secreted (RANTES), which contributes to the emergence and maintenance of cancer stem-like cells, thereby driving tumorigenesis [160]. Another study revealed that SASP factors including IL-6 and IL-8 can promote EMT, a crucial process in tumor invasion and metastasis [161]. Con-

versely, there is also evidence indicating that some SASP factors, such as IL-15 and C-X-C motif chemokine ligand 1 (CXCL1), acts as tumor suppressors by recruiting immune cells like natural killer cells and neutrophils to eliminate tumor cells [162]. These findings underscore the multifaceted and context-dependent roles of senescent cells and their altered lysosomal functions in tumor progression, highlighting their potential dual role in both promoting and suppressing tumorigenesis.

7. Clinical Applications Targeting the Lysosomal-Autophagic Pathway for Cancer Treatment

The lysosomal-autophagic pathway plays a complex role in tumor progression, but its clinical applications have seen notable advancements. Chloroquine (CQ) and its derivative hydroxychloroquine (HCQ) are autophagy inhibitors known to block lysosomal acidification and autophagosome processing [163,164]. In preclinical and animal studies, CQ and HCQ have shown ability to prevent chemotherapy resistance for various cancers [165]. As clinical-approved autophagy inhibitors, CQ and HCQ had been undergoing clinical evaluation for cancer therapy [163,166]. For instance, a Phase I trial investigating the combination of HCQ with temozolomide in melanoma patients showed that the treatment was safe and tolerable [167]. Another Phase I trial observed that combining CQ or HCQ with carboplatin and gemcitabine improved survival in patients with advanced solid tumors [168]. Beyond CQ and HCQ, other autophagy inhibitors have been identified, targeting different stages of the process. Examples include SBI-0206965 and NSC185058, which target unc-51-like kinase 1 (ULK1) and ATG4B, respectively [169,170]. Taken together, these advancements indicate that therapeutic interventions targeting different stages of autophagy could play a crucial role in developing precision medicine for various cancer types.

8. Conclusion & Perspective

Lysosomes, often regarded as cell's 'garbage bags', are central to cellular degradation and play a vital role in maintaining cellular homeostasis. Beyond their degradative function, lysosomes are essential regulators of various signaling pathways, including nutrient sensing, metabolic signaling, and iron metabolism. They mediate energy metabolism, cell proliferation and differentiation, cell senescence, and cell death through various mechanisms. In cancer biology, lysosomal-autophagic pathway plays a dual role. It suppresses tumor initiation by degrading mutated proteins, thereby maintaining cellular integrity. However, it can also promote tumor cell growth and proliferation by supplying nutrients and energy. Suppression of autophagy by mTORC1 can lead to energy crisis and trigger apoptosis in cancer cells [171]. Additionally, studies have shown that lysosomes can promote tumor cell invasion and metastasis.

Understanding the specific roles of lysosomal-autophagy pathway at different stage of tumor development is crucial for developing targeted interventions in tumor therapy. Notably, in recent years, increasing evidence has demonstrated that lysosomes can influence how cancer cells interact with the immune system, suggesting significant potential for applications in tumor immunotherapy [172]. Lysosomes have been shown to be major site for the degradation of immune checkpoint proteins, such as programmed death-1 (PD-1), PD-L1, and cytotoxic T-lymphocyte associated protein 4 (CTLA4), whose blockade has displayed promising clinical effects in tumor immunotherapy [172,173]. Combining lysosome inhibitors with immune checkpoint inhibitors holds potential for enhancing anti-tumor efficacy. For example, a recent study shows that the chemical Luminespib (AUY-922) can promote lysosomal degradation of PD-L1, and improving the anti-tumor effectiveness of anti-PD-L1 and anti-CTLA4 treatments [174]. Another study reveals that the natural marine product benzoesceptin C (BC) can also trigger lysosome-mediated degradation of PD-L1, with the combination of BC with anti-CTLA significantly boosting therapeutic outcomes [175]. Overall, these findings highlight that intervening lysosome could be a promising strategy for improving the efficacy of cancer immunotherapy.

Author Contributions

YFL, FHX and XQC conceptualized the review. XQC, QY, and WMC drafted the manuscript. XQC, QY, and WMC analyzed and interpreted the reviewed studies. ZWC, GHG, XZ, XMS and TS performed the literature searches. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Fennelly C, Amaravadi RK. Lysosomal Biology in Cancer. In Öllinger K, Appelqvist H (eds.) Lysosomes (pp. 293–308). Springer New York: New York. 2017.
- [2] Davidson SM, Vander Heiden MG. Critical Functions of the Lysosome in Cancer Biology. Annual Review of Pharmacology and Toxicology. 2017; 57: 481–507.
- [3] Schröder BA, Wrocklage C, Hasilik A, Saftig P. The proteome of lysosomes. Proteomics. 2010; 10: 4053–4076.
- [4] Trivedi PC, Bartlett JJ, Pulinilkunnil T. Lysosomal Biology and Function: Modern View of Cellular Debris Bin. Cells. 2020; 9: 1131.
- [5] Xiong J, Zhu MX. Regulation of lysosomal ion homeostasis by channels and transporters. Science China. Life Sciences. 2016; 59: 777–791.
- [6] Mindell JA. Lysosomal acidification mechanisms. Annual Review of Physiology. 2012; 74: 69–86.
- [7] Lloyd-Evans E, Waller-Evans H. Lysosomal Ca^{2+} Homeostasis and Signaling in Health and Disease. Cold Spring Harbor Perspectives in Biology. 2020; 12: a035311.
- [8] Settembre C, Fraldi A, Medina DL, Ballabio A. Signals from the lysosome: a control centre for cellular clearance and energy metabolism. Nature Reviews. Molecular Cell Biology. 2013; 14: 283–296.
- [9] Nowosad A, Besson A. Lysosomes at the Crossroads of Cell Metabolism, Cell Cycle, and Stemness. International Journal of Molecular Sciences. 2022; 23: 2290.
- [10] Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell. 2004; 119: 753–766.
- [11] Yu T, Zuber J, Li J. Targeting autophagy in skin diseases. Journal of Molecular Medicine (Berlin, Germany). 2015; 93: 31–38.
- [12] Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. Nature. 2011; 469: 323–335.
- [13] Lawrence RE, Zoncu R. The lysosome as a cellular centre for signalling, metabolism and quality control. Nature Cell Biology. 2019; 21: 133–142.
- [14] Perera RM, Zoncu R. The Lysosome as a Regulatory Hub. Annual Review of Cell and Developmental Biology. 2016; 32: 223–253.
- [15] Panwar V, Singh A, Bhatt M, Tonk RK, Azizov S, Raza AS, *et al.* Multifaceted role of mTOR (mammalian target of rapamycin) signaling pathway in human health and disease. Signal Transduction and Targeted Therapy. 2023; 8: 375.
- [16] Cao M, Luo X, Wu K, He X. Targeting lysosomes in human disease: from basic research to clinical applications. Signal Transduction and Targeted Therapy. 2021; 6: 379.
- [17] Guo F, Liu X, Cai H, Le W. Autophagy in neurodegenerative diseases: pathogenesis and therapy. Brain Pathology (Zurich, Switzerland). 2018; 28: 3–13.
- [18] Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. Nature Reviews. Cancer. 2017; 17: 528–542.
- [19] Li J, Zhang D, Wiersma M, Brundel BJM. Role of Autophagy in Proteostasis: Friend and Foe in Cardiac Diseases. Cells. 2018; 7: 279.
- [20] Zhang P, Zhu J, Zhang L, Lv X, Guo D, Liao L, *et al.* The Effects of *Ginkgo biloba* Extract on Autophagy in Human Macrophages Stimulated by Cigarette Smoke Extract. Frontiers in Bioscience (Landmark Edition). 2023; 28: 50.
- [21] Mizushima N. Autophagy in protein and organelle turnover. Cold Spring Harbor Symposia on Quantitative Biology. 2011; 76: 397–402.
- [22] Das G, Shravage BV, Baehrecke EH. Regulation and function of autophagy during cell survival and cell death. Cold Spring Harbor Perspectives in Biology. 2012; 4: a008813.
- [23] Tooze SA, Dikic I. Autophagy Captures the Nobel Prize. Cell. 2016; 167: 1433–1435.
- [24] Zhao MM, Wang RS, Zhou YL, Yang ZG. Emerging relationship between RNA helicases and autophagy. Journal of Zhejiang University. Science. B. 2020; 21: 767–778.
- [25] Bai Z, Peng Y, Ye X, Liu Z, Li Y, Ma L. Autophagy and cancer treatment: four functional forms of autophagy and their therapeutic applications. Journal of Zhejiang University. Science. B. 2022; 23: 89–101.
- [26] Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. Cell. 2011; 147: 728–741.
- [27] Reggiori F, Ungermann C. Autophagosome Maturation and Fusion. Journal of Molecular Biology. 2017; 429: 486–496.
- [28] Zachari M, Ganley IG. The mammalian ULK1 complex and autophagy initiation. Essays in Biochemistry. 2017; 61: 585–596.
- [29] Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. Annual Review of Cell and Developmental Biology. 2011; 27: 107–132.
- [30] Hurley JH, Young LN. Mechanisms of Autophagy Initiation. Annual Review of Biochemistry. 2017; 86: 225–244.
- [31] Chang C, Jensen LE, Hurley JH. Autophagosome biogenesis comes out of the black box. Nature Cell Biology. 2021; 23: 450–456.
- [32] Hanada T, Noda NN, Satomi Y, Ichimura Y, Fujioka Y, Takao T, *et al.* The Atg12-Atg5 conjugate has a novel E3-like activity for protein lipidation in autophagy. The Journal of Biological Chemistry. 2007; 282: 37298–37302.
- [33] Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, *et al.* A ubiquitin-like system mediates protein lipidation. Nature. 2000; 408: 488–492.
- [34] Gatica D, Lahiri V, Klionsky DJ. Cargo recognition and degradation by selective autophagy. Nature Cell Biology. 2018; 20: 233–242.
- [35] Feng Y, He D, Yao Z, Klionsky DJ. The machinery of macroautophagy. Cell Research. 2014; 24: 24–41.
- [36] Wang L, Klionsky DJ, Shen HM. The emerging mechanisms and functions of microautophagy. Nature Reviews. Molecular Cell Biology. 2023; 24: 186–203.
- [37] Oku M, Sakai Y. Three Distinct Types of Microautophagy Based on Membrane Dynamics and Molecular Machineries. BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology. 2018; 40: e1800008.
- [38] Dice JF. Chaperone-mediated autophagy. Autophagy. 2007; 3: 295–299.
- [39] Dolezal P, Likic V, Tachezy J, Lithgow T. Evolution of the molecular machines for protein import into mitochondria. Science (New York, N.Y.). 2006; 313: 314–318.
- [40] Park C, Suh Y, Cuervo AM. Regulated degradation of Chk1 by chaperone-mediated autophagy in response to DNA damage. Nature Communications. 2015; 6: 6823.
- [41] Kiffin R, Christian C, Knecht E, Cuervo AM. Activation of chaperone-mediated autophagy during oxidative stress. Molecular Biology of the Cell. 2004; 15: 4829–4840.
- [42] Auzmendi-Iriarte J, Otaegi-Ugartemendia M, Carrasco-Garcia E, Azkargorta M, Diaz A, Saenz-Antoñanzas A, *et al.* Chaperone-Mediated Autophagy Controls Proteomic and Transcriptomic Pathways to Maintain Glioma Stem Cell Activity. Cancer Research. 2022; 82: 1283–1297.
- [43] Qin ZH. Autophagy: Biology and Diseases: Basic Science. Springer Singapore: Singapore. 2019.
- [44] Kaushik S, Cuervo AM. The coming of age of chaperone-mediated autophagy. Nature Reviews. Molecular Cell Biology.

2018; 19: 365–381.

- [45] Bandyopadhyay U, Kaushik S, Varticovski L, Cuervo AM. The chaperone-mediated autophagy receptor organizes in dynamic protein complexes at the lysosomal membrane. *Molecular and Cellular Biology*. 2008; 28: 5747–5763.
- [46] Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell*. 2010; 141: 290–303.
- [47] Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell*. 2007; 12: 9–22.
- [48] Menon S, Dibble CC, Talbott G, Hoxhaj G, Valvezan AJ, Takahashi H, *et al.* Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. *Cell*. 2014; 156: 771–785.
- [49] Bar-Peled L, Sabatini DM. Regulation of mTORC1 by amino acids. *Trends in Cell Biology*. 2014; 24: 400–406.
- [50] Kodiha M, Rassi JG, Brown CM, Stochaj U. Localization of AMP kinase is regulated by stress, cell density, and signaling through the MEK→ERK1/2 pathway. *American Journal of Physiology. Cell Physiology*. 2007; 293: C1427–C1436.
- [51] Carroll B, Dunlop EA. The lysosome: a crucial hub for AMPK and mTORC1 signalling. *The Biochemical Journal*. 2017; 474: 1453–1466.
- [52] Jia J, Bissa B, Brecht L, Allers L, Choi SW, Gu Y, *et al.* AMPK is activated during lysosomal damage via a galectin-ubiquitin signal transduction system. *Autophagy*. 2020; 16: 1550–1552.
- [53] Hardie DG. AMPK and autophagy get connected. *The EMBO Journal*. 2011; 30: 634–635.
- [54] Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nature Cell Biology*. 2011; 13: 132–141.
- [55] Luzio JP, Pryor PR, Bright NA. Lysosomes: fusion and function. *Nature Reviews. Molecular Cell Biology*. 2007; 8: 622–632.
- [56] Morgan AJ, Platt FM, Lloyd-Evans E, Galione A. Molecular mechanisms of endolysosomal Ca²⁺ signalling in health and disease. *The Biochemical Journal*. 2011; 439: 349–374.
- [57] Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nature Reviews. Molecular Cell Biology*. 2000; 1: 11–21.
- [58] Ballabio A, Bonifacino JS. Lysosomes as dynamic regulators of cell and organismal homeostasis. *Nature Reviews. Molecular Cell Biology*. 2020; 21: 101–118.
- [59] Nguyen HT, Wiedeker A, Wollheim CB, Park KS. Regulation of autophagy by perilyosomal calcium: a new player in β -cell lipotoxicity. *Experimental & Molecular Medicine*. 2024; 56: 273–288.
- [60] Cheng X, Shen D, Samie M, Xu H. Mucopolins: Intracellular TRPML1-3 channels. *FEBS Letters*. 2010; 584: 2013–2021.
- [61] Xu H, Ren D. Lysosomal physiology. *Annual Review of Physiology*. 2015; 77: 57–80.
- [62] Kim HJ, Soyombo AA, Tjon-Kon-Sang S, So I, Muallem S. The Ca(2+) channel TRPML3 regulates membrane trafficking and autophagy. *Traffic (Copenhagen, Denmark)*. 2009; 10: 1157–1167.
- [63] Brailoiu E, Churamani D, Cai X, Schrlau MG, Brailoiu GC, Gao X, *et al.* Essential requirement for two-pore channel 1 in NAADP-mediated calcium signaling. *The Journal of Cell Biology*. 2009; 186: 201–209.
- [64] Lu Y, Hao B, Graeff R, Yue J. NAADP/TPC2/Ca(2+) Signaling Inhibits Autophagy. *Communicative & Integrative Biology*. 2013; 6: e27595.
- [65] Fairbairn IP, Stober CB, Kumararatne DS, Lammas DA. ATP-mediated killing of intracellular mycobacteria by macrophages is a P2X(7)-dependent process inducing bacterial death by phagosome-lysosome fusion. *Journal of Immunology (Baltimore, Md.: 1950)*. 2001; 167: 3300–3307.
- [66] Qureshi OS, Paramasivam A, Yu JCH, Murrell-Lagnado RD. Regulation of P2X4 receptors by lysosomal targeting, glycan protection and exocytosis. *Journal of Cell Science*. 2007; 120: 3838–3849.
- [67] Astaburuaga R, Quintanar Haro OD, Stauber T, Relógio A. A Mathematical Model of Lysosomal Ion Homeostasis Points to Differential Effects of Cl[−] Transport in Ca²⁺ Dynamics. *Cells*. 2019; 8: 1263.
- [68] Wang S, Tsun ZY, Wolfson RL, Shen K, Wyant GA, Plovnick ME, *et al.* Metabolism. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. *Science (New York, N.Y.)*. 2015; 347: 188–194.
- [69] Mackenzie B, Erickson JD. Sodium-coupled neutral amino acid (System N/A) transporters of the SLC38 gene family. *Pflügers Archiv: European Journal of Physiology*. 2004; 447: 784–795.
- [70] Cao Q, Zhong XZ, Zou Y, Zhang Z, Toro L, Dong XP. BK Channels Alleviate Lysosomal Storage Diseases by Providing Positive Feedback Regulation of Lysosomal Ca²⁺ Release. *Developmental Cell*. 2015; 33: 427–441.
- [71] Kasper D, Planells-Cases R, Fuhrmann JC, Scheel O, Zeitz O, Ruether K, *et al.* Loss of the chloride channel ClC-7 leads to lysosomal storage disease and neurodegeneration. *The EMBO Journal*. 2005; 24: 1079–1091.
- [72] Lange PF, Wartosch L, Jentsch TJ, Fuhrmann JC. ClC-7 requires Ostm1 as a beta-subunit to support bone resorption and lysosomal function. *Nature*. 2006; 440: 220–223.
- [73] Graves AR, Curran PK, Smith CL, Mindell JA. The Cl[−]/H⁺ antiporter ClC-7 is the primary chloride permeation pathway in lysosomes. *Nature*. 2008; 453: 788–792.
- [74] Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, *et al.* Ferroptosis: an iron-dependent form of non-apoptotic cell death. *Cell*. 2012; 149: 1060–1072.
- [75] Yu Z, Persson HL, Eaton JW, Brunk UT. Intralysosomal iron: a major determinant of oxidant-induced cell death. *Free Radical Biology & Medicine*. 2003; 34: 1243–1252.
- [76] Terman A, Kurz T. Lysosomal iron, iron chelation, and cell death. *Antioxidants & Redox Signaling*. 2013; 18: 888–898.
- [77] Palmiter RD, Cole TB, Findley SD. ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. *The EMBO Journal*. 1996; 15: 1784–1791.
- [78] McCormick NH, Kelleher SL. ZnT4 provides zinc to zinc-dependent proteins in the trans-Golgi network critical for cell function and Zn export in mammary epithelial cells. *American Journal of Physiology. Cell Physiology*. 2012; 303: C291–C297.
- [79] Choi KS. Autophagy and cancer. *Experimental & Molecular Medicine*. 2012; 44: 109–120.
- [80] White E. Deconvoluting the context-dependent role for autophagy in cancer. *Nature Reviews. Cancer*. 2012; 12: 401–410.
- [81] Singh SS, Vats S, Chia AYQ, Tan TZ, Deng S, Ong MS, *et al.* Dual role of autophagy in hallmarks of cancer. *Oncogene*. 2018; 37: 1142–1158.
- [82] Dutzler R. Structural basis for ion conduction and gating in ClC chloride channels. *FEBS Letters*. 2004; 564: 229–233.
- [83] Poroca DR, Pelis RM, Chappe VM. ClC Channels and Transporters: Structure, Physiological Functions, and Implications in Human Chloride Channelopathies. *Frontiers in Pharmacology*. 2017; 8: 151.
- [84] Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. *Nature Reviews. Cancer*. 2007; 7: 961–967.
- [85] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, *et al.* Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature*. 1999; 402: 672–676.
- [86] Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, *et al.* Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *The Journal of Clinical Investigation*.

- 2003; 112: 1809–1820.
- [87] Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100: 15077–15082.
 - [88] Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, *et al.* Autophagy-deficient mice develop multiple liver tumors. *Genes & Development*. 2011; 25: 795–800.
 - [89] Murphy MP. How mitochondria produce reactive oxygen species. *The Biochemical Journal*. 2009; 417: 1–13.
 - [90] Huang R, Chen H, Liang J, Li Y, Yang J, Luo C, *et al.* Dual Role of Reactive Oxygen Species and their Application in Cancer Therapy. *Journal of Cancer*. 2021; 12: 5543–5561.
 - [91] Liu B, He G. Role of Autophagy in Cancer Development via Mitochondrial Reactive Oxygen Species. *Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging* (pp. 215–229). Elsevier: Amsterdam. 2016.
 - [92] Poillet-Perez L, Despouy G, Delage-Mourroux R, Boyer-Guittaut M. Interplay between ROS and autophagy in cancer cells, from tumor initiation to cancer therapy. *Redox Biology*. 2015; 4: 184–192.
 - [93] White E, Mehnert JM, Chan CS. Autophagy, Metabolism, and Cancer. *Clinical Cancer Research: an Official Journal of the American Association for Cancer Research*. 2015; 21: 5037–5046.
 - [94] Zhi F, Li B, Zhang C, Xia F, Wang R, Xie W, *et al.* NLRP6 potentiates PI3K/AKT signalling by promoting autophagic degradation of p85 α to drive tumorigenesis. *Nature Communications*. 2023; 14: 6069.
 - [95] Marei HE, Althani A, Afifi N, Hasan A, Caceci T, Pozzoli G, *et al.* p53 signaling in cancer progression and therapy. *Cancer Cell International*. 2021; 21: 703.
 - [96] Vakifahmetoglu-Norberg H, Kim M, Xia HG, Iwanicki MP, Ofengeim D, Coloff JL, *et al.* Chaperone-mediated autophagy degrades mutant p53. *Genes & Development*. 2013; 27: 1718–1730.
 - [97] Fujii S, Mitsunaga S, Yamazaki M, Hasebe T, Ishii G, Kojima M, *et al.* Autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. *Cancer Science*. 2008; 99: 1813–1819.
 - [98] Debnath J, Gammoh N, Ryan KM. Autophagy and autophagy-related pathways in cancer. *Nature Reviews. Molecular Cell Biology*. 2023; 24: 560–575.
 - [99] Mathew R, White E. Autophagy, stress, and cancer metabolism: what doesn't kill you makes you stronger. *Cold Spring Harbor Symposia on Quantitative Biology*. 2011; 76: 389–396.
 - [100] Luo T, Fu J, Xu A, Su B, Ren Y, Li N, *et al.* PSMD10/gankyrin induces autophagy to promote tumor progression through cytoplasmic interaction with ATG7 and nuclear transactivation of ATG7 expression. *Autophagy*. 2016; 12: 1355–1371.
 - [101] Liu M, Jiang L, Fu X, Wang W, Ma J, Tian T, *et al.* Cytoplasmic liver kinase B1 promotes the growth of human lung adenocarcinoma by enhancing autophagy. *Cancer Science*. 2018; 109: 3055–3067.
 - [102] White E, DiPaola RS. The double-edged sword of autophagy modulation in cancer. *Clinical Cancer Research: an Official Journal of the American Association for Cancer Research*. 2009; 15: 5308–5316.
 - [103] Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, *et al.* Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell*. 2006; 10: 51–64.
 - [104] Marquez RT, Xu L. Bcl-2:Beclin 1 complex: multiple, mechanisms regulating autophagy/apoptosis toggle switch. *American Journal of Cancer Research*. 2012; 2: 214–221.
 - [105] Rabinowitz JD, White E. Autophagy and metabolism. *Science* (New York, N.Y.). 2010; 330: 1344–1348.
 - [106] Liu EY, Ryan KM. Autophagy and cancer—issues we need to digest. *Journal of Cell Science*. 2012; 125: 2349–2358.
 - [107] Lorin S, Pattingre S, Meijer AJ, Codogno P. Regulation of Macroautophagy by Nutrients and Metabolites. *The Molecular Nutrition of Amino Acids and Proteins* (pp. 181–203). Elsevier: Amsterdam. 2016.
 - [108] Meijer AJ, Codogno P. Autophagy: regulation and role in disease. *Critical Reviews in Clinical Laboratory Sciences*. 2009; 46: 210–240.
 - [109] Guo JY, Xia B, White E. Autophagy-mediated tumor promotion. *Cell*. 2013; 155: 1216–1219.
 - [110] Kania E, Pająk B, Orzechowski A. Calcium homeostasis and ER stress in control of autophagy in cancer cells. *BioMed Research International*. 2015; 2015: 352794.
 - [111] Boya P, Reggiori F, Codogno P. Emerging regulation and functions of autophagy. *Nature Cell Biology*. 2013; 15: 713–720.
 - [112] Wei H, Wei S, Gan B, Peng X, Zou W, Guan JL. Suppression of autophagy by FIP200 deletion inhibits mammary tumorigenesis. *Genes & Development*. 2011; 25: 1510–1527.
 - [113] Zhu D, Zhou J, Zhao J, Jiang G, Zhang X, Zhang Y, *et al.* ZC3H13 suppresses colorectal cancer proliferation and invasion via inactivating Ras-ERK signaling. *Journal of Cellular Physiology*. 2019; 234: 8899–8907.
 - [114] Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, *et al.* Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes & Development*. 2011; 25: 460–470.
 - [115] Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. *Nature Reviews. Molecular Cell Biology*. 2008; 9: 517–531.
 - [116] Goel S, Huang J, Klampfer L. K-Ras, intestinal homeostasis and colon cancer. *Current Clinical Pharmacology*. 2015; 10: 73–81.
 - [117] Masliah-Planchon J, Garinet S, Pasmant E. RAS-MAPK pathway epigenetic activation in cancer: miRNAs in action. *Oncotarget*. 2016; 7: 38892–38907.
 - [118] Lippai M, Löw P. The role of the selective adaptor p62 and ubiquitin-like proteins in autophagy. *BioMed Research International*. 2014; 2014: 832704.
 - [119] Lim J, Lachenmayer ML, Wu S, Liu W, Kundu M, Wang R, *et al.* Proteotoxic stress induces phosphorylation of p62/SQSTM1 by ULK1 to regulate selective autophagic clearance of protein aggregates. *PLoS Genetics*. 2015; 11: e1004987.
 - [120] Fan L, Yin S, Zhang E, Hu H. Role of p62 in the regulation of cell death induction. *Apoptosis: an International Journal on Programmed Cell Death*. 2018; 23: 187–193.
 - [121] Duran A, Linares JF, Galvez AS, Wikenheiser K, Flores JM, Diaz-Meco MT, *et al.* The signaling adaptor p62 is an important NF-kappaB mediator in tumorigenesis. *Cancer Cell*. 2008; 13: 343–354.
 - [122] Inami Y, Waguri S, Sakamoto A, Kouno T, Nakada K, Hino O, *et al.* Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *The Journal of Cell Biology*. 2011; 193: 275–284.
 - [123] Kenific CM, Thorburn A, Debnath J. Autophagy and metastasis: another double-edged sword. *Current Opinion in Cell Biology*. 2010; 22: 241–245.
 - [124] Hamurcu Z, Delibaşı N, Geçene S, Şener EF, Dönmez-Altuntaş H, Özkul Y, *et al.* Targeting LC3 and Beclin-1 autophagy genes suppresses proliferation, survival, migration and invasion by inhibition of Cyclin-D1 and uPAR/Integrin β 1/ Src signaling in triple negative breast cancer cells. *Journal of Cancer Research and Clinical Oncology*. 2018; 144: 415–430.
 - [125] Hashimoto I, Koizumi K, Tatematsu M, Minami T, Cho S, Takeno N, *et al.* Blocking on the CXCR4/mTOR signalling path-

- way induces the anti-metastatic properties and autophagic cell death in peritoneal disseminated gastric cancer cells. *European Journal of Cancer* (Oxford, England: 1990). 2008; 44: 1022–1029.
- [126] Yun CW, Lee SH. The Roles of Autophagy in Cancer. *International Journal of Molecular Sciences*. 2018; 19: 3466.
- [127] Kenific CM, Stehbens SJ, Goldsmith J, Leidal AM, Faure N, Ye J, *et al*. NBR1 enables autophagy-dependent focal adhesion turnover. *The Journal of Cell Biology*. 2016; 212: 577–590.
- [128] Sharifi MN, Mowers EE, Drake LE, Collier C, Chen H, Zamora M, *et al*. Autophagy Promotes Focal Adhesion Disassembly and Cell Motility of Metastatic Tumor Cells through the Direct Interaction of Paxillin with LC3. *Cell Reports*. 2016; 15: 1660–1672.
- [129] Lock R, Kenific CM, Leidal AM, Salas E, Debnath J. Autophagy-dependent production of secreted factors facilitates oncogenic RAS-driven invasion. *Cancer Discovery*. 2014; 4: 466–479.
- [130] Marsh T, Tolani B, Debnath J. The pleiotropic functions of autophagy in metastasis. *Journal of Cell Science*. 2021; 134: jcs247056.
- [131] Wei H, Wang C, Croce CM, Guan JL. p62/SQSTM1 synergizes with autophagy for tumor growth in vivo. *Genes & Development*. 2014; 28: 1204–1216.
- [132] Morris VL, Tuck AB, Wilson SM, Percy D, Chambers AF. Tumor progression and metastasis in murine D2 hyperplastic alveolar nodule mammary tumor cell lines. *Clinical & Experimental Metastasis*. 1993; 11: 103–112.
- [133] La Belle Flynn A, Calhoun BC, Sharma A, Chang JC, Almasan A, Schiemann WP. Autophagy inhibition elicits emergence from metastatic dormancy by inducing and stabilizing Pfkfb3 expression. *Nature Communications*. 2019; 10: 3668.
- [134] Marsh T, Kenific CM, Suresh D, Gonzalez H, Shamir ER, Mei W, *et al*. Autophagic Degradation of NBR1 Restricts Metastatic Outgrowth during Mammary Tumor Progression. *Developmental Cell*. 2020; 52: 591–604.e6.
- [135] Qiang L, He YY. Autophagy deficiency stabilizes TWIST1 to promote epithelial-mesenchymal transition. *Autophagy*. 2014; 10: 1864–1865.
- [136] Duprez L, Wirawan E, Vanden Berghe T, Vandenabeele P. Major cell death pathways at a glance. *Microbes and Infection*. 2009; 11: 1050–1062.
- [137] Yu F, Chen Z, Wang B, Jin Z, Hou Y, Ma S, *et al*. The role of lysosome in cell death regulation. *Tumour Biology: the Journal of the International Society for Oncodevelopmental Biology and Medicine*. 2016; 37: 1427–1436.
- [138] Johansson AC, Appelqvist H, Nilsson C, Kågedal K, Roberg K, Ollinger K. Regulation of apoptosis-associated lysosomal membrane permeabilization. *Apoptosis: an International Journal on Programmed Cell Death*. 2010; 15: 527–540.
- [139] Mimikjoo B, Balasubramanian K, Schroit AJ. Mobilization of lysosomal calcium regulates the externalization of phosphatidylserine during apoptosis. *The Journal of Biological Chemistry*. 2009; 284: 6918–6923.
- [140] Dauer P, Gupta VK, McGinn O, Nomura A, Sharma NS, Arora N, *et al*. Inhibition of Sp1 prevents ER homeostasis and causes cell death by lysosomal membrane permeabilization in pancreatic cancer. *Scientific Reports*. 2017; 7: 1564.
- [141] Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, *et al*. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death and Differentiation*. 2012; 19: 107–120.
- [142] Alu A, Han X, Ma X, Wu M, Wei Y, Wei X. The role of lysosome in regulated necrosis. *Acta Pharmaceutica Sinica. B*. 2020; 10: 1880–1903.
- [143] Kallunki T, Olsen OD, Jäättelä M. Cancer-associated lysosomal changes: friends or foes? *Oncogene*. 2013; 32: 1995–2004.
- [144] Liu S, Perez P, Sun X, Chen K, Fatirikhori R, Mammadova J, *et al*. MLKL polymerization-induced lysosomal membrane permeabilization promotes necroptosis. *Cell Death and Differentiation*. 2024; 31: 40–52.
- [145] Lu L, Zhang Y, Tan X, Merkhher Y, Leonov S, Zhu L, *et al*. Emerging mechanisms of pyroptosis and its therapeutic strategy in cancer. *Cell Death Discovery*. 2022; 8: 338.
- [146] Chu X, Xiao X, Wang G, Uosef A, Lou X, Arnold P, *et al*. Gasdermin D-mediated pyroptosis is regulated by AMPK-mediated phosphorylation in tumor cells. *Cell Death & Disease*. 2023; 14: 469.
- [147] Bergsbaken T, Fink SL, den Hartigh AB, Loomis WP, Cookson BT. Coordinated host responses during pyroptosis: caspase-1-dependent lysosome exocytosis and inflammatory cytokine maturation. *Journal of Immunology* (Baltimore, Md.: 1950). 2011; 187: 2748–2754.
- [148] Bruchard M, Mignot G, Derangère V, Chalmin F, Chevriaux A, Végran F, *et al*. Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the Nlrp3 inflammasome and promotes tumor growth. *Nature Medicine*. 2013; 19: 57–64.
- [149] Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, *et al*. Ferroptosis: process and function. *Cell Death and Differentiation*. 2016; 23: 369–379.
- [150] Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, *et al*. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell*. 2017; 171: 273–285.
- [151] Rizzollo F, More S, Vangheluwe P, Agostinis P. The lysosome as a master regulator of iron metabolism. *Trends in Biochemical Sciences*. 2021; 46: 960–975.
- [152] Chen X, Kang R, Kroemer G, Tang D. Organelle-specific regulation of ferroptosis. *Cell Death and Differentiation*. 2021; 28: 2843–2856.
- [153] Fan C, Wu H, Du X, Li C, Zeng W, Qu L, *et al*. Inhibition of lysosomal TRPML1 channel eliminates breast cancer stem cells by triggering ferroptosis. *Cell Death Discovery*. 2024; 10: 256.
- [154] Gao H, Bai Y, Jia Y, Zhao Y, Kang R, Tang D, *et al*. Ferroptosis is a lysosomal cell death process. *Biochemical and Biophysical Research Communications*. 2018; 503: 1550–1556.
- [155] Wu Z, Geng Y, Lu X, Shi Y, Wu G, Zhang M, *et al*. Chaperone-mediated autophagy is involved in the execution of ferroptosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2019; 116: 2996–3005.
- [156] Swanda RV, Ji Q, Wu X, Yan J, Dong L, Mao Y, *et al*. Lysosomal cystine governs ferroptosis sensitivity in cancer via cysteine stress response. *Molecular Cell*. 2023; 83: 3347–3359.e9.
- [157] Park JT, Lee YS, Cho KA, Park SC. Adjustment of the lysosomal-mitochondrial axis for control of cellular senescence. *Ageing Research Reviews*. 2018; 47: 176–182.
- [158] Ohtani N. The roles and mechanisms of senescence-associated secretory phenotype (SASP): can it be controlled by senolysis? *Inflammation and Regeneration*. 2022; 42: 11.
- [159] Dong Z, Luo Y, Yuan Z, Tian Y, Jin T, Xu F. Cellular senescence and SASP in tumor progression and therapeutic opportunities. *Molecular Cancer*. 2024; 23: 181.
- [160] Cahu J, Bustany S, Sola B. Senescence-associated secretory phenotype favors the emergence of cancer stem-like cells. *Cell Death & Disease*. 2012; 3: e446.
- [161] Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, *et al*. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biology*. 2008; 6: 2853–2868.
- [162] Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, *et al*. Senescence and tumour clearance is trig-

- gered by p53 restoration in murine liver carcinomas. *Nature*. 2007; 445: 656–660.
- [163] Wang Y, Peng RQ, Li DD, Ding Y, Wu XQ, Zeng YX, *et al.* Chloroquine enhances the cytotoxicity of topotecan by inhibiting autophagy in lung cancer cells. *Chinese Journal of Cancer*. 2011; 30: 690–700.
- [164] Pellegrini P, Strambi A, Zipoli C, Hägg-Olofsson M, Buoncervello M, Linder S, *et al.* Acidic extracellular pH neutralizes the autophagy-inhibiting activity of chloroquine: implications for cancer therapies. *Autophagy*. 2014; 10: 562–571.
- [165] Sui X, Chen R, Wang Z, Huang Z, Kong N, Zhang M, *et al.* Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment. *Cell Death & Disease*. 2013; 4: e838.
- [166] Lim J, Murthy A. Targeting Autophagy to Treat Cancer: Challenges and Opportunities. *Frontiers in Pharmacology*. 2020; 11: 590344.
- [167] Rangwala R, Leone R, Chang YC, Fecher LA, Schuchter LM, Kramer A, *et al.* Phase I trial of hydroxychloroquine with dose-intense temozolomide in patients with advanced solid tumors and melanoma. *Autophagy*. 2014; 10: 1369–1379.
- [168] Karim NA, Ullah A, Ahmad I, Bahassi E, Olowokure O, Khaled A, *et al.* A Phase I Trial to Determine the Safety and Tolerability of Autophagy Inhibition Using Chloroquine or Hydroxychloroquine in Combination with Carboplatin and Gemcitabine in Patients With Advanced Solid Tumors. *Frontiers in Oncology*. 2022; 12: 811411.
- [169] Pasquier B. SAR405, a PIK3C3/Vps34 inhibitor that prevents autophagy and synergizes with MTOR inhibition in tumor cells. *Autophagy*. 2015; 11: 725–726.
- [170] Akin D, Wang SK, Habibzadegah-Tari P, Law B, Ostrov D, Li M, *et al.* A novel ATG4B antagonist inhibits autophagy and has a negative impact on osteosarcoma tumors. *Autophagy*. 2014; 10: 2021–2035.
- [171] Gremke N, Polo P, Dort A, Schneikert J, Elmshäuser S, Brehm C, *et al.* mTOR-mediated cancer drug resistance suppresses autophagy and generates a druggable metabolic vulnerability. *Nature Communications*. 2020; 11: 4684.
- [172] Xu Y, Shao B, Zhang Y. The significance of targeting lysosomes in cancer immunotherapy. *Frontiers in Immunology*. 2024; 15: 1308070.
- [173] Butterfield LH, Najjar YG. Immunotherapy combination approaches: mechanisms, biomarkers and clinical observations. *Nature Reviews. Immunology*. 2024; 24: 399–416.
- [174] Xu X, Xie T, Zhou M, Sun Y, Wang F, Tian Y, *et al.* Hsc70 promotes anti-tumor immunity by targeting PD-L1 for lysosomal degradation. *Nature Communications*. 2024; 15: 4237.
- [175] Wang Q, Wang J, Yu D, Zhang Q, Hu H, Xu M, *et al.* Benzosceptrin C induces lysosomal degradation of PD-L1 and promotes antitumor immunity by targeting DHHC3. *Cell Reports. Medicine*. 2024; 5: 101357.