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Lysosomal Biology in Cancer

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Abstract

Cells depend on the lysosome for sequestration and degradation of macromolecules in order to maintain metabolic homeostasis. These membrane-enclosed organelles can receive intracellular and extracellular cargo through endocytosis, phagocytosis, and autophagy. Lysosomes establish acidic environments to activate enzymes that are able to break down biomolecules engulfed through these various pathways. Recent advances in methods to study the lysosome have allowed the discovery of extended roles for the lysosome in various diseases, including cancer, making it an attractive and targetable node for therapeutic intervention. This review focuses on key aspects of lysosomal biology in the context of cancer and how these properties can be exploited for the development of new therapeutic strategies. This will provide a contextual framework for how advances in methodology could be applied in future translational research.

Keywords

Lysosome; Cancer; Drug therapy; Autophagy

1 Introduction to the Lysosome

The lysosome is a membrane-enclosed organelle that functions as an essential part of the digestive system of the cell. Christian de Duve first discovered lysosomes in 1955 and the name was derived from the Greek term for digestive body. This organelle contains over 60 different types of hydrolases that can break down biological polymers such as proteins, carbohydrates, lipids, and nucleic acids [1]. These enzymes require an acidic pH for optimal function, which is achieved by using ATP hydrolysis to pump protons against their electrochemical gradient into the lysosome by the vacuolar H⁺ ATPase (V-ATPase) [2, 3]. Due to their pH dependence, these enzymes are also called acid hydrolases. They are produced in the endoplasmic reticulum (ER), trafficked to the golgi apparatus and tagged with mannose-6-phosphate to be targeted to the lysosome [4]. Vesicular exchange between the trans-golgi network (TGN) and endosomes is involved in the transport of newly synthesized proteins from the TGN to the endolysosomal compartment, and the reverse, where some proteins are trafficked from the endolysosomal compartment back to the TGN. Trafficking vesicles are categorized by their internal pH, where early endosomes are around 6–6.6, late endosomes are at 5 and lysosomes are the most acidic at pH 4.5. The mannose-6-phosphate receptor marker is used to identify endosomes and lysosomes. Intracellular cargo is delivered to the lysosomes through autophagy, whereas exogenous material can be engulfed from outside the cell and delivered via endocytosis or phagocytosis. Endocytosis occurs in either a clathrin-dependent or -independent manner. The lysosome serves as the end organelle for these degradative endocytic pathways that begin at the plasma membrane.

Once lysosomal substrates are broken down, their components can be recycled and reused by the cell as building blocks for macromolecules through various carrier-mediated transport channels back into the cytosol [5, 6]. Other than the ubiquitin-proteasome system, autophagy is the main degradation pathway for intracellular proteins. Unlike proteasomal degradation, autophagy can accommodate organelles and cytoskeletal components in addition to proteins. During chaperone-mediated autophagy, cytosolic proteins that contain specific motifs are localized to the lysosome through the action of chaperones and the lysosomal receptor LAMP-2A [7–9]. Another form of autophagy is microautophagy, which involves the direct engulfment of cytoplasmic cargo at the lysosomal membrane [10]. The most widely studied form of autophagy is macroautophagy, and therefore, hereafter our discussion of autophagy will focus on this form. Autophagic degradation is accomplished through the sequestration of soluble cargo into a double membrane structure—referred to as a phagophore—to form an autophagosome that eventually fuses with the lysosome to complete the degradation process [11]. In addition to the main autophagy pathway, there are the more recently recognized organelle-specific autophagy processes of lysophagy [12, 13], mitophagy [14], ER-phagy [15], nucleophagy [16], and pexophagy [17]. This review will outline how lysosomal biogenesis is regulated, our current understanding of the many roles lysosomes play in cancer progression and cell death, examples of tool compounds that can be used to modulate lysosomal function (Fig. 1), and a brief overview of efforts to translate some of these findings into clinical trials.

2 Control of Lysosomal Biogenesis at the Transcriptional Level

Lysosome formation is typically thought of in terms of simply the vesicular trafficking of key lysosomal proteins from the ER, golgi, endosomes, and eventually into lysosomes. However, recent evidence suggests that lysosomal biogenesis is coordinated at the transcriptional level in a sophisticated manner, and can even play a critical role in cancer cell metabolism [18, 19]. Transcription factor EB (TFEB) is a transcription factor that acts as a master regulator for lysosomal biogenesis and drives the expression of over 500 genes related to autophagy and autophagosome-lysosome fusion [20]. Other family members of the TFE/MiTF family control this expression profile in different cellular contexts. Activation of this expression profile called the CLEAR (coordinated lysosomal expression and regulation) network occurs when TFEB translocates from the lysosomal membrane into the nucleus. This system controls the expression of lysosomal enzymes required for the breakdown of biomolecules and genes linked to the main trafficking pathways including autophagy, endo/exocytosis, and phagocytosis [21]. Recent work using unbiased global metabolite profiling revealed the MiT/TFE family critically supports the metabolism of pancreatic ductal adenocarcinoma (PDA) [18]. The discovery of this expression profile for lysosomal biogenesis opens the door to new biomarkers and therapeutic targets.

3 Lysosomes and Cancer Progression

Besides its role in catabolism and recycling—i.e. feeding the cancer cell from the inside—recent evidence indicates the lysosome is also a central node for metabolic growth signaling. Cancer cells deviate from normal metabolism in order to acquire their idiosyncratic feature of uncontrolled growth. This transformation results in rapid depletion of cellular nutrients,

accumulation of aggregated proteins, and damaged organelles making certain cancer cells dependent on lysosomal recycling programs for survival and continued growth. Autophagic-lysosomal degradation of macromolecules and organelles serves as a coping mechanism for cancer cells to deal with these stresses while also providing a consistent supply of nutrients to promote further growth. Additionally, lysosomes are not just degradative vesicles, but signaling scaffolds for mTOR and AMPK signaling, as described later. They are arguably the main nutrient sensing organelle in the cell. Targeting lysosomes can have pleiotropic effects involving metabolism [22], reactive oxygen species (ROS) [23], DNA damage [24], cell death [25, 26], and protein secretion [27].

Cancer cells depend on lysosome function and demonstrate changes in lysosomal volume and subcellular localization during oncogenic transformation [28, 29]. Cathepsin proteases are lysosomal hydrolases that can play dual roles in promoting and suppressing tumor growth. They are observed as being upregulated and mislocalized in cancer [29, 30]. Intracellular cathepsins are able to activate the intrinsic apoptotic pathway, but in contrast, extracellular cathepsins promote tumor invasion through their ability to break down basement membranes and activate other oncogenic proteins. In addition, cathepsins B, E, and S have all been recognized as contributing to malignancy in different cancers [31–33]. Lysosomal membrane proteins like lysosome-associated membrane protein 1 (LAMP-1) have been observed on the cell surface of highly metastatic colon cells, indicating a role for these proteins in the extracellular matrix [34]. Other lysosomal membrane proteins such as the V-ATPase have been shown to exert an influence on the tumor microenvironment by pumping protons to the extracellular space [35]. The Na⁺/H⁺ exchanger has also been associated with extracellular acidification and cancer cell invasion [36]. Another intriguing aspect of lysosomes is their ability to secrete contents out of the cell by fusing with the plasma membrane [4, 37]. For example, cells can expel ATP to the extracellular space with this secretory pathway to mediate cellular signaling through ATP receptors [38, 39]. The observation that lysosomal exocytosis can play a role in cell signaling, proteolytic extracellular matrix (ECM) remodeling and tumor invasion suggests that targeting lysosomal exocytosis rather than individual cathepsins would be a more promising strategy [40].

The lysosome is an important signaling hub that responds to both external and internal stimuli to perceive the availability of nutrients, growth factor signals, and energy to maintain metabolic homeostasis. One of the main regulators of cell growth and proliferation is mammalian target of rapamycin complex 1 (mTORC1), which exerts its function directly from the lysosomal membrane surface. mTORC1 is a multicomponent protein kinase complex that includes mTOR, Regulatory Associated Protein of mTOR (RAPTOR), and mLST8/GβL [41]. mTORC1 and its regulatory complexes detailed below, together integrate various nutritional and environmental cues including the presence of amino acids, growth factors, glucose, hormones, and oxygen to drive anabolic processes such as protein, mRNA, and lipid biosynthesis [22, 42, 43]. Active mTORC1 also phosphorylates ULK1 and ATG13 to inhibit their activity and block autophagy [44]. Additionally, TFEB has been recognized as a target for mTORC1 suggesting this interaction directly influences expression of the CLEAR network genes [45]. This ability to control biosynthetic and catabolic states makes mTORC1 an important factor in metabolic signaling and mutations that lead to defective mTORC1 regulation are commonly observed in human cancers [41, 46]. Oncogenic

transformation is significantly enabled by mutations that lead to inactivation of key tumor suppressor genes including phosphatase and tensin homolog (PTEN), tuberous sclerosis complex 1/2 (TSC1/TSC2), neurofibromin 1/2 (NF1/NF2), and liver kinase B1 (LKB1). In all of these cases the downstream consequence of this inactivation is promotion of mTORC1 signaling [47]. mTORC1 kinase activity is stimulated by direct interaction with the GTPase Ras homolog enriched in brain (RHEB) on the lysosome surface [48]. This interaction is negatively regulated by the heterodimer TSC1/TSC2 and promoted by amino acids that recruit mTORC1 to the lysosomal surface through Rag GTPases that are stabilized by the Regulator complex [49]. Recent investigations have also revealed that the V-ATPase can mediate mTORC1 activation and autophagy [50]. This provides further evidence that mTORC1 localization to the lysosomal surface is essential for its activation. Molecular sensors for amino acids (Rags), growth factor inputs (Rheb), energy status (LKB1/AMPK1), and lysosomal health (V-ATPase) all have to be aligned for full activation of mTORC1.

Loss of the tumor suppressor PTEN has been shown to activate mTORC1 via protein kinase B (AKT), which inhibits TSC1/TSC2 through phosphorylation [41]. TSC1/TSC2 can also be suppressed when LKB1 is inactivated, preventing TSC1/TSC2 phosphorylation and activation [41]. LKB1 regulates AMPK directly, and recent evidence indicates that AMPK is also closely associated with the lysosomal surface. Another example of mTORC1-driven oncogenesis is activation of eukaryotic translation initiation factor 4B (eIF4E) through mTORC1-mediated inhibition of 4EBP1 [51]. This results in mRNA translation of cell cycle regulatory genes and pro-tumorigenic genes such as the anti-apoptotic protein Mcl-1 that can promote cancer cell survival in in vivo mouse models of lymphoma [52–54].

The lysosome's role in catabolic recycling and metabolic growth decisions suggests there may be therapeutic potential in targeting the lysosome. Great progress has been made in understanding the cell fates associated with lysosomal targeting, i.e. the role of the lysosome in eliciting cell death.

4 Lysosomes and Cell Death

Lysosomes can play a role in each of the three major types of cancer cell death that include apoptosis, autophagy, or necrosis [55]. A more recent form of cell death ferroptosis is also dependent on the lysosome [56]. For apoptosis there are intrinsic and extrinsic pathways that can be activated by different mechanisms. The intrinsic pathway involves mitochondrial outer membrane permeabilization (MOMP) and cytochrome c release into the cytoplasm, whereas the extrinsic is initiated by cell death receptors [57]. Both result in caspase signaling cascades that are governed by the Bcl-2 family of proteins that ultimately regulate MOMP. These proteins are classified in two categories: antiapoptotic (e.g. Bcl-2 and Bcl-xL) and proapoptotic (e.g. Bax and Bid) [58]. Damaged lysosomes allow proteolytic enzymes to be released into the cytosol and initiate apoptosis [59]. Cathepsins B and D are known to cleave Bid when ectopically in the cytosol, which results in MOMP followed by cytochrome c release [60].

Cancer metabolism can create harsh byproducts such as ammonia, ROS, and hypoxia [61–64]. To sustain oncogenic growth and cell survival autophagy can play a cytoprotective role

that counteracts apoptosis by intercepting damaged mitochondria that could trigger apoptosis [65, 66]. Autophagy can have dual roles in the context of cancer and is also recognized as a cell death pathway [67]. High drug doses can initiate apoptosis-independent and autophagy-dependent cell death in vitro, although the relevance of autophagic cell death in vivo has been called into question [68]. Necroptosis is a form of programmed necrosis that serves as a backup role when apoptosis signaling is blocked by endogenous or exogenous factors including viruses or mutations. The receptor-interacting serine/threonine protein kinase 1 (RIPK1) and RIPK3 have been identified as regulators of apoptosis and necrosis. RIPK1/3 can induce necroptosis via sphingomyelinase-mediated lysosomal membrane permeabilization (LMP) [69]. Ferroptosis is an additional and unique form of cell death that is dependent on iron and ROS [56]. It is distinct in that it has its own morphological, genetic, and biochemical signatures. Misregulation of iron metabolism and lipid peroxidation has been implicated in various pathologies including cancer [70, 71].

One common theme that can impact multiple cell death pathways is LMP. This process has been the topic of intense study for decades, and steadily methods to measure LMP in cancer cells have become more reproducible and versatile. LMP permits the release of lysosomal hydrolases into the cytosol and can contribute to the forms of cell death discussed above [28, 60]. Depending on the degree of permeabilization, LMP can either induce lysosomal cell death through apoptosis or necrosis if the subsequent enzyme release is extensive enough [72, 73]. For instance, LMP can initiate caspase cascades via the intrinsic apoptosis pathway through cleavage of Bid and induction of Bax-mediated release of cytochrome c, but cathepsins are able to mediate cell death in a caspase-independent manner as well [74]. Identifying stimuli that can cause the release of these lysosomal enzymes such as cathepsins into the cytosolic lumen has potential applications for targeting the lysosome in cancer. LMP can be evaluated in cells by detecting functional enzyme activity of lysosomal hydrolases present in the cytosol or visually by either tracking lysosomes with fluorescent dextran or staining with antibody probes against galectins [75]. A small molecule screen done to identify compounds able to induce p53-independent cell death found that the ones that were effective worked through an LMP mechanism [76]. These findings and other studies have lead to the proposal that transformed cells are more sensitive to lysosomal cell death and further support the notion that targeting the lysosome can be an effective therapeutic strategy [77].

5 Drugs That Target the Lysosome

There are at least five categories of drugs that target the lysosome. These include lysosomal hydrolase inhibitors [78–82], heat shock protein 70 (HSP70) inhibitors, cationic amphiphilic compounds, V-ATPase inhibitors, and chloroquine derivatives that do not yet have a clear mechanism of action.

Acid sphingomyelinase (ASM) is located in the lysosome and breaks down sphingomyelin into ceramide, which is the substrate for the generation of other sphingolipids including sphingomyelin and sphingosine 1-phosphate (S1P) [83]. It has been observed that cancer cells display decreased levels of the proapoptotic lipid ceramide and increased levels of proliferation promoting lipid S1P [84]. Cancer cells also exhibit lower ASM activity leading

to higher sphingomyelin levels. Blocking ASM activity has shown to further elevate sphingomyelin levels and interrupt the function of the lysosomal membrane [85]. HSP70 is expressed in many tumor types and can activate ASM, which is associated with increased lysosomal integrity. Targeting HSP70 thereby inactivating ASM with small molecule inhibitors like 2-phenylethanesulfonamide (PES) can increase LMP and cause cell death [86, 87]. Other drugs such as chloroquine (CQ), chlorpromazine, and amiodarone are cationic amphiphilic agents that displace ASM from vesicular membranes in the lysosome and result in lysosomal membrane permeabilization (LMP) and eventual tumor cell death [86].

HSP70 promotes tumor cell metastasis and survival by protecting lysosomal membrane integrity. It can serve as a biomarker for poor prognosis due to its higher expression in many cancers. The HSP70 modulator PES disrupts the protein interaction with p53 resulting in massive accumulation of autophagosomes loaded with undigested cargo and cellular apoptosis [88].

Bafilomycin A1 is the prototypical inhibitor of the V-ATPase and prevents lysosomal acidification and autophagic flux. It is similar to other compounds that are of microbial origin including archazolid and cleistanthin A. However, other mechanisms of action have been proposed for bafilomycin's effects on the lysosome and autophagy. Bafilomycin A1 has also been shown to prevent autophagosome formation by activation of mTOR signaling, suggesting that it may target both the early and late stages of autophagy [89]. This impairment is mediated by dissociation of the Beclin1-Vps34 complex and encourages Bcl-2 interaction to drive autophagy inhibition and apoptotic cell death [37]. Interestingly, Bafilomycin has also been shown to engage the mitochondria and induce translocation of apoptosis inducing factor to the nucleus and provoke caspase-independent apoptosis [90]. Archazolid is another V-ATPase inhibitor and myxobacterial agent that has shown the ability to reduce the activity of the protease cathepsin B both in vitro and in vivo [91]. A member of the manzamine alkaloids, manzamine A, was isolated from marine sponges of the genus *Haliclona*, and demonstrated to have inhibiting effects on autophagy and the V-ATPase in pancreatic cancer cells [92]. The diphyllin glycoside cleistanthin-A also has cytotoxic effects on various tumor cell lines and targets the V-ATPase [93].

Salinomycin is a monocarboxylic polyether antibiotic that was isolated from a *Streptomyces albus* strain and functions as an ionophore in the lysosome to facilitate the transport of cations across cellular membranes (including lysosomal) [94–96]. Salinomycin has been shown to impair autophagic flux in breast cancer cells [97] and even act in concert with Gefitinib to induce apoptosis in colorectal cancer cells [98]. For the latter, this process was dependent on ROS production and lead to loss of mitochondrial outer membrane potential and LMP. Other groups have also recognized oxidative stress as an important factor in salinomycin-induced cell growth inhibition in prostate cancer cells [99]. Co-treatment of salinomycin with doxorubicin or etoposide led to DNA damage and apoptosis in drug-resistant cancer cells. This was also associated with enhanced expression of p53 and H2AX as well as concurrent reduction in p21 [100]. In a different study, salinomycin suppressed elevated p21 resulting from radiation treatment and promoted activation of H2AX and p53 resulting in DNA damage and G2 arrest [101]. Salinomycin is suggested to have selective cytotoxic effects on cancer stem cells and also sensitize tumor cells to conventional

chemotherapeutic drugs including methotrexate, adriamycin, and cisplatin in vitro and in vivo [102].

CQ accumulates in lysosomes and blocks autophagy by disrupting acidification and enzyme function [103]. However, a definitive mechanism for CQ in mammalian cells remains elusive. Other weak base compounds are known to also accumulate in lysosomes [104], but none are known to inhibit autophagy. Interestingly, other drugs have been observed to accumulate in lysosomes and it has been suggested that this mitigates the cytotoxic effect of these compounds and aids in drug resistance [105–108]. A series of novel monomeric CQ derivatives were tested in both lung and pancreatic cancer cells and proved to be eightfold more potent than CQ [109]. Other efforts have identified the antimalarial agent quinacrine (QN) as being much more effective at autophagy inhibition than CQ [110]. The synthesis of novel monomeric QN analogs led to the generation of improved lysomotropic agents that targeted the lysosome and elicited cell death in various cancer cell types in vitro [110]. Another lysosomal agent, lucanthone, has been reported to inhibit lysosomal function and induce apoptosis in a p53-independent manner [111]. Interestingly, these effects appear to be dependent on cathepsin D. However, this agent has been suggested to block topoisomerase II activity and inhibits AP endonuclease (APE1), an important enzyme in DNA base excision repair suggesting it may not be specific for the lysosome [112].

6 Clinical Trials and Future Outlook

Over 40 clinical trials using hydroxychloroquine (HCQ) are being conducted worldwide in humans and dogs [113]. Six phase I/II clinical trials have been performed in patients diagnosed with refractory myeloma, glioblastoma, melanoma, and other cancers [114–119]. These trials also include combination therapies that were designed from preclinical studies [120–124]. Clinical trials to date have demonstrated that autophagy inhibition could be achieved safely in patients. This was concluded from evidence of accumulated autophagic vesicles in peripheral blood mononuclear cells and tumor cells. Even though high doses were required to achieve this effect, treatment combinations were generally well tolerated and there were not any signs of liver damage, metabolic dysfunction, or neurological impairment [113]. However, there were some HCQ-cancer drug combinations that did result in dose-limiting toxicities. In phase II clinical trials, patients previously treated for metastatic pancreatic cancer were given HCQ alone and high doses were tolerated, but did not demonstrate high therapeutic efficacy [119]. This suggests more potent compounds are needed to generate the desired outcomes with the overall strategy of autophagy inhibition.

One of the limitations for clinical trials is biomarker availability for assessment of drug efficacy. In the case of the autophagy inhibitor HCQ, the current methods include EM visualization of autophagic vesicle accumulation in peripheral blood mononuclear cells and tumor cells along with LC3 western blotting and evaluation of total LC3 with immunohistochemistry. Studies have been done to characterize secreted factors of tumor cells exhibiting high autophagy and indicate that these could be potential candidates for biomarkers [27]. There is growing evidence to encourage the concept that more potent autophagy inhibitors could eventually be used synergistically with conventional chemotherapy or radiotherapy [125]. Recent work has led to the dimerization of CQ to

generate a CQ derivative (Lys05), which has proven to be far more potent as a single agent *in vivo* and in combination with B-Raf proto-oncogene serine/threonine protein kinase (BRAF) inhibitors [126, 127]. Future studies will need to expand on the findings to date to further elucidate the role of lysosomal function in tumor biology. Fortunately, there are many opportunities to elicit an effect on lysosomal activity involving factors related to nutrient sensing, kinase signaling, death signaling, and cell trafficking. Coupling functional studies and molecular biology techniques will confirm the identification of new target candidates and potential biomarkers. Capitalizing on other approaches involving high-throughput readouts to analyze patient samples could also help detect what aspects of human cancer is prone to lysosomal inhibition and lead to new clinical therapies.

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Fig. 1.

The key elements involved in lysosomal biology in cancer. Factors that can play roles in cancer progression are in red. Vesicular trafficking funnels cellular contents into the lysosome from various pathways including endocytosis and autophagy. TFEB regulates expression of autophagy and lysosomal genes. Elevated autophagy has proven to aid in oncogenesis by providing macromolecules for sustained growth and eliminating damaged proteins, organelles, and hazardous waste including ammonia and ROS. Cellular contents can also be secreted to the extracellular space through exocytosis. Lysosomal cathepsins have been observed in the extracellular space where they are free to engage the ECM for remodeling to promote tumor invasion. Lysosomal membrane proteins such as the V-ATPase and LAMP2 have also been observed promoting metastasis by exerting an influence on the tumor microenvironment from the plasma membrane. MTORC1 is known to positively regulate growth and feed into other oncogenic signaling pathways. HSP70 supports lysosomal membrane integrity by activating ASM and can even be used as a biomarker for prognosis. Various drugs (*green*) that target the lysosome or cellular components involved with the lysosome can destabilize the organelle leading to LMP and activation of cell death pathways (*yellow*). *aa* amino acid, *ASM* acid sphingomyelinase, *Baf* Bafilomycin A 1, *CQ* chloroquine, *ECM* extracellular matrix, *HSP70* 70-kDa heat shock proteins, *LAMP* lysosome associated membrane glycoproteins, *LMP* lysosomal membrane permeabilization, *mTORC1* mammalian target of rapamycin complex 1/mechanistic target of rapamycin complex 1, *PES* phenylethynesulfonamide, *QN* quinacrine, *ROS* reactive oxygen species, *TFEB* transcription factor EB, *V-ATPase* Vacuolar-type H⁺ATPase