

Role of lysosomes in cancer therapy

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Abstract: Lysosomes are acidic organelles that are involved in cellular digestion by endocytosis, phagocytosis, and autophagy. They contain more than 50 hydrolases that are capable of degrading all macromolecules. There is accumulating evidence that lysosomal enzymes can provoke apoptotic cell death. This has important implications for cancer, where proapoptotic genes are mutated and antiapoptotic genes are often overexpressed leading to chemoresistance. Lysosomes play a dual role in cancer development depending on their subcellular localization. When they are located extracellularly they can promote invasion, angiogenesis, and metastasis. However, when they are located intracellularly they can trigger apoptosis by leaking into the cytosol. In this review, we examine the pathways by which lysosomes can evoke both apoptosis and tumorigenesis. Although cancer cells have defects in their apoptotic machinery, they can still undergo lysosomal cell death. We offer several strategies to explain how targeting lysosomes can serve as a putative model for the development of novel anticancer agents. Furthermore, we propose that lysosomal cell death is an effective treatment against apoptosis-resistant cancer cells and thus holds great potential as a therapeutic strategy for circumventing apoptosis deficiency in tumors.

Keywords: cathepsins, lysosomal membrane permeability, apoptosis, chemoresistance

Introduction

Lysosomes, discovered more than 50 years ago, are the major cell-digestive organelles that degrade extracellular materials taken up by the cell and intracellular components under certain conditions.^{1,2} They contain a number of hydrolases that are capable of breaking down nucleic acids, proteins, carbohydrates, and lipids. However, it is becoming more evident that these organelles function more in the cellular digestion process. It has taken some time to recognize that lysosomal proteases play a role in programmed cell death (PCD) for the following reasons. Some inhibitors that were used to evaluate the role of caspases in PCD also inhibit several cathepsins that function as effectors of lysosomal cell death.^{3,4} Furthermore, lysosomal involvement in PCD has been overlooked because the morphology of the lysosomal membrane often appears intact even after proteases have translocated into the cytosol.⁵ Today, it is clear that lysosomes and lysosomal proteases are involved in apoptosis. Lysosomal cell death or type II cell death⁶ is characterized by early lysosomal destruction of the cytoplasm with nuclear degradation being a late event. In type II cell death, a ladder of fragmented DNA may be generated, but only after much of the cytoplasm has already been destroyed.⁷ Nuclear changes are often delayed and the cytosol is consumed by autophagic vacuoles in lysosomal cell death.^{7,8} Type II death is exemplified during

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normal development by the regression of the tadpole tail,⁹ involuting mammary gland,¹⁰ involuting prostate,¹¹ and dying insect tissues.^{7,12,13} One of the first reports that demonstrated that lysosomotropic agents destabilized the lysosomal membrane triggering apoptosis was conducted by Li et al.¹⁴ Subsequently, several other studies have reported that agents that directly disrupt the lysosomal membrane confirmed that lysosomal membrane permeabilization (LMP) can trigger apoptosis and apoptosis-like cell death.^{15–17}

Lysosomes

Lysosomes are the organelles that are responsible for cellular digestion, which were identified by Appelmans et al.¹⁸ and de Duve et al.¹⁹ Figure 1 summarizes the various functions carried out by lysosomes in normal and cancer cells. Lysosomes perform cellular degradation by utilizing more than 50 hydrolases, including proteases, glycosidases, lipases, and nucleases.²⁰ As a result of this wide range of enzymes, lysosomes can degrade all types of macromolecules. The hydrolases are active under acidic pH, which is maintained by the action of the vacuolar H⁺-ATPase.²¹ The lysosomes are also involved in endocytosis,²² exocytosis,²³ cholesterol homeostasis,²⁴ chaperone-mediated autophagy,²⁵ and macroautophagy.²⁶ Figure 1 also demonstrates the dual role played by lysosomes in cancer cells. Lysosomal proteases that are secreted extracellularly can promote tumorigenesis.^{27–29} Overexpression of lysosomal proteases often correlates with poor prognosis and increased recurrence in many cancers.^{30,31} By contrast, cytosolic release of lysosomal enzymes has been demonstrated to trigger apoptosis and cell death.^{3,15,32}

Mounting evidence indicate that lysosomal enzymes are good target molecules for cancer therapy.^{29,33,34}

Lysosomes in cancer cells

Cancer cells have relatively large lysosomes³⁵ and these are thought to be more fragile than normal-sized lysosomes.³⁶ Moreover, cancer cells exhibit higher metabolic rates and an increased turnover of iron-containing proteins, leading to the lysosomal accumulation of iron, with consequent iron-mediated sensitization to reactive oxygen species (ROS)-induced LMP.³⁷ Cancer cells often produce elevated ROS levels and the associated higher rate of spontaneous cathepsin release from lysosomes may facilitate cell death induction.³⁸ On theoretical grounds, all of these factors render lysosomes from cancer cells particularly susceptible to the therapeutic induction of lysosomal-mediated cell death. Further studies are needed to confirm these observations. The presence of lysosomal-mediated cell death provides a uniquely useful option to treat tumor cells that are resistant to canonical apoptotic cell death. We propose that cancer cells are inherently more susceptible to lysosomal-mediated cell death than normal cells and that this very characteristic should be fully explored to design more effective antitumor agents. Furthermore, the enhancement of lysosomal cell death may be a therapeutic strategy to overcome inhibition to caspase-dependent cell death.

In contrast, cancer cells try to evade cell death by making modifications that affect their lysosomes. The increased activity of phosphatidylinositol-3-kinase (PI3K), which is characteristic of many tumors, may contribute to the

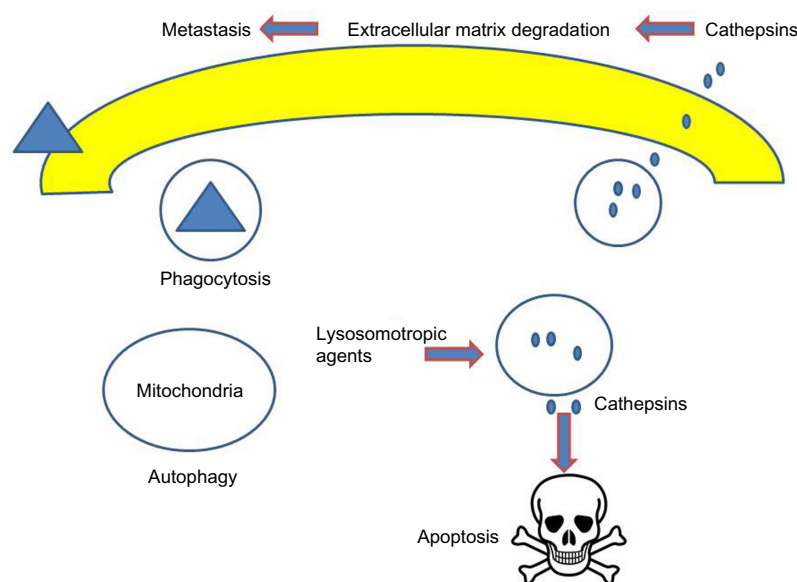


Figure 1 Various functions of lysosomes in normal and cancer cells.

stability of tumor cell lysosomes.³³ PI3K regulates several cell processes, including maturation, size, and activity of lysosomes,³⁹ and the inhibition of PI3K can shift the TNF-induced cell death pathway from caspase dependent to cathepsin dependent.⁴⁰ Cancer cells have developed mechanisms to inhibit LMP by overexpression of lysosomal cytosolic protease inhibitors.^{41,42} Furthermore, cancer cells translocate cytosolic Hsp70 to the lysosomal lumen where it stabilizes the lysosomal membranes by promoting the activity of acid sphingomyelinase.^{43,44} Tumor cells appear to protect themselves against cytosolic leakage of lysosomal proteases by translocation of Hsp70 into the lysosomal membrane.⁴⁴ Depletion of Hsp70 triggers a tumor-cell-specific lysosomal cell death program.³⁸ Finally, cathepsin inhibitors may increase the viability of cancer cells with enhanced lysosomal activity.^{45,46} Therefore, it is conceivable that drugs that inhibit the activity of PI3K, the lysosomal accumulation of Hsp70, or the activity of cathepsin inhibitors may make tumor cells susceptible to lysosomal cell death.

Lysosomes and cancer progression

In addition to the role of lysosomal enzymes in triggering LMP, translocation of enzymes from the lumen of lysosomes to the cytosol, and triggering mitochondrial-mediated apoptosis, they have been documented to promote tumorigenesis. Cathepsins have been documented to promote tumors as well as acting as tumor suppressors depending on whether they are released intracellularly where they induce apoptosis or released into the extracellular matrix (ECM) where they stimulate metastasis.³² Malignant cells display increased proteolytic activity, which helps them to digest the ECM. Cathepsins have been shown to degrade the ECM components, such as elastin, laminin, and fibronectin, which facilitate invasion, angiogenesis, and metastasis.⁴⁷⁻⁴⁹ Upregulation of cysteine cathepsin protein levels has been detected in mouse and human cancers.^{49,50} Furthermore, there is a correlation between the expression levels of these proteins and clinical outcome.^{49,51-53} In some instances, lysosomal proteases are translocated to the plasma membrane and then secreted into the ECM.^{54,55} Using null mutants for cysteine cathepsin genes, it has been demonstrated that these genes contribute to pancreatic islet tumorigenesis.⁵⁶ The exact mechanisms by which cathepsins promote their pro-tumor effects have yet to be fully elucidated.

Increased lysosomal enzymatic activity and secretion of the hydrolases into the extracellular space promotes tumor growth and migration, angiogenesis, and metastasis.^{27,57,58} Several members of the cathepsin family of proteases have

been implicated in tumor progression. In particular, protein expression levels of cathepsins B, D, and L have been shown to increase.⁵⁹ There is often a redistribution of lysosomes from perinuclear to peripheral locations in cancer cells and the lysosomal contents have been localized to the extracellular space.⁶⁰ There are data supporting the premise that secreted cathepsins, working in collaboration with matrix metalloproteases and the plasminogen activator system, can degrade the ECM, thereby promoting cellular motility, invasion, and angiogenesis.^{50,61}

Lysosomes and cell death in cancer

The role of cathepsins in apoptosis is well documented.^{2,62,63} Cathepsins have been implicated as TNF- α -induced apoptosis of murine fibrosarcoma WEHI-S cells³ and ovarian cancer OV-90 cells.⁶⁴ Lysosomal cathepsins B and D modulate apoptosis involving cytotoxic drugs.^{65,66} One report showed that selective alteration of the lysosomal membrane with L-leucyl-L-leucine methyl ester resulted in apoptosis in HeLa cells that was characterized by translocation of cathepsins into the cytosol and cleavage of Bid.¹⁶ Lysosomes contain an activity capable of cleaving Bid.^{67,68} However, lysosomal hydrolases can trigger the intrinsic apoptotic pathway independent of Bid cleavage.¹⁵ LMP has been proposed as the primary mechanism responsible for the release of lysosomal proteases into the cytosol.^{63,69} Once released into the cytosol, cysteine cathepsins can induce liberation of cytochrome c from the mitochondria and subsequent activation of caspases.⁶⁷ Lysosomal proteases migrate from the lysosomal lumen to the cytosol in response to various apoptotic stimuli such as TNF,³ Fas,⁷⁰ p53 activation,⁷¹ microtubule-stabilizing agents,⁶⁵ oxidative stress,⁷² and lysosomotropic agents.¹⁵

The exact mechanisms by which LMP modulates apoptosis are not well understood. It has been proposed that caspase-8 is involved in the release of apoptotic factors from lysosomes.¹⁶ Another report demonstrated that triptolide sensitizes TRAIL-resistant pancreatic cancer cells to apoptosis by inducing LMP.⁷³ Translocation of cathepsins into the cytosol occurs as a result of LMP. Lysosomal-mediated cell death serves as an important alternative death pathway for tumors that acquire chemoresistance via mutations in proapoptotic genes, such as executioner caspases, or anti-apoptotic genes, such as *XIAP* and *Bcl-2*.

Mechanisms of LMP

There are several terms that are currently used to describe the process that results in the translocation of lysosomal

proteases from the lysosomal lumen to the cytosol: lysosomal membrane destabilization, lysosomal membrane disruption, and LMP. There is some confusion as to how to accurately detect lysosomal membrane alterations and when to use these terms. It is generally accepted that they all involve damage to the lysosomal membrane. To further propagate the confusion, the structure of the lysosomal membrane can appear to be intact even after lysosomal proteases have leaked into the cytosol.⁵ Lysosomal membrane destabilization has been observed in hydrogen-peroxide-treated cells that displayed deformed and swollen lysosomes.⁷⁴ Another study demonstrated lysosomal membrane destabilization in neuronal cells by detecting a loss in lysosomal proton gradient integrity.⁷⁵ However, several agents have been shown to directly disrupt the integrity of the lysosomal membrane.^{16,17,72,76} A quantitative relationship between the amount of damage to the lysosomal membrane and the mode of cell death may explain the different morphological outcomes following LMP. Limited release of lysosomal proteases into the cytosol results in apoptosis-like cell death, while massive rupture of the lysosomal membrane leads to necrosis.^{17,72} However, the exact mechanisms that regulate the translocation of lysosomal proteases into the cytosol remain unknown, and further studies are required to achieve a better understanding of this process.

The precise mechanisms responsible for lysosomal rupture are not yet completely understood. It is not known whether pores form in the lysosomal membrane or whether certain channels are involved in the relocation of lysosomal proteases from the lysosomal lumen to the cytosol. However, one can propose several plausible explanations. The presence of lysosomotropic agents, such as sphingosine, could accumulate in lysosomes, permeabilize lysosomal membranes, and facilitate the leakage of some lysosomal proteases.¹⁷ Downregulation of Hsp70 has been demonstrated to result in lysosomal destabilization, release of lysosomal hydrolases into the cytosol, and a caspase-independent cell death.⁴⁴ Another possible explanation for provoking lysosomal rupture involves the production of ROS. Specifically, several stimuli that induce lysosomal leakage, such as TNF- α ,⁷⁷ also generate the production of H₂O₂ during apoptosis.^{78,79} An alternate possibility is that proapoptotic proteins, such as Bax, may translocate into the lysosomal membrane and trigger lysosomal rupture.⁸⁰ Photodynamic therapy has also been reported to induce lysosomal disruption, resulting in mitochondria-mediated cell death.⁸¹

Following their release into the cytosol, cathepsins cleave Bid and degrade antiapoptotic Bcl-2 proteins, thereby triggering the mitochondrial pathway of apoptosis, with LMP being the critical step in this pathway.^{63,82} Regardless of the

trigger of LMP, it is generally accepted that cytosolic release of cathepsins precedes mitochondrial-membrane potential changes.^{17,68,83} How LMP is modulated by the complex Bcl-2 protein network, however, is still unclear. Various insults, including oxidative stress and DNA damage, may lead to the limited release of cathepsins that culminate in the induction of apoptosis.⁸⁴⁻⁸⁷ Hsp70 plays an important role in inhibiting LMP to promote the survival of stressed cells.⁴³ However, blocking cathepsins by small-molecule inhibitors has been shown to significantly delay cancer progression in a number of mouse cancer models as well as to sensitize tumor cells to other chemotherapeutic agents.⁸⁸ The promoter region of cathepsin D contains two p53-binding sites and the authors concluded that cells that completely lack this protease exhibited increased chemoresistance.⁸⁹ Lysosomal-mediated apoptosis is still largely under investigation and not fully understood.

Targeting lysosomes in anticancer therapy

Lysosomotropic agents

Drugs that are weak bases can accumulate in acidic organelles, such as lysosomes by an interaction described as the acidic shift.⁹⁰ The accumulation of these basic drugs in lysosomes, which occurs because of the pH gradient across the lysosomal membrane and ion trapping, can result in cytosolic leakage of lysosomal hydrolases and cell death.^{91,92} To this end, Firestone et al designed a class of agents termed lysosomotropic detergents to treat cancer cells.⁹³ This was demonstrated by showing that lysosomal entrapment of the imidazoacridinone C1311 resulted in lysosomal swelling and rupture followed by subsequent cytosolic release of proteases and cell death of HT-29 colon cancer cells.⁹⁴ The use of lysosomotropic compounds as anticancer agents has increased. Lysosomes and lysosomal enzymes have also been postulated to play a role in the shrinkage of solid tumors.^{95,96,97} Furthermore, it has been described that the efficacy of radiation⁹⁶ and hyperthermia⁹⁷ are due, in part, to lysosomal enzymatic degradation and cytosolic leakage of these proteases. Mefloquine, an antimalarial agent, has been shown to possess antileukemic activity.⁹⁸ In this study, the authors demonstrated that the size of lysosomes were increased in acute myeloid leukemia cells and that mefloquine was toxic to acute myeloid leukemia cells while sparing normal hematopoietic cells treated with the same doses.⁹⁸

The majority of chemotherapeutic drugs induce cell death via the mitochondrial-mediated intrinsic apoptotic pathway. Accumulating data indicate that cathepsins are located upstream of mitochondrial outer membrane permeabilization in this signaling pathway and can provoke mitochondrial

outer membrane permeabilization.^{66,99} It was reported that a cathepsin B inhibitor reduced cell death and cytochrome c release in embelin (an inhibitor of XIAP)-treated colon cancer cells.¹⁰⁰ This finding indicates that the lysosomal cell death pathway is upstream of mitochondrial death signaling. Other studies have demonstrated that lysosomal-mediated cell death in cancer cells can be caspase-independent. Resveratrol has been shown to activate apoptosis by promoting lysosome leakage and cytosolic translocation of cathepsin D in colorectal cancer cells.¹⁰¹ We have previously demonstrated that the Chinese herb triptolide, the bioactive component of *Tripterygium wilfordii* Hook f, induces upregulation of the mature, active form of cathepsin B protein levels in cytosolic fractions of MCF-7 cells.¹⁰² Using LysoTracker Green, we detected an aggregation of lysosomes in experimental cells, but not in control cells.¹⁰² This lends support to the notion that LMP and cytosolic leakage of lysosomal proteases are upstream of mitochondrial changes in the intrinsic apoptotic pathway.^{32,103} Furthermore, when MCF-7 cells were stained with acridine orange, a fluorescent dye that accumulates in acidic organelles, control cells displayed distinct red fluorescence (lysosomal) while triptolide-treated cells displayed reduced red fluorescence and maximal green fluorescence (cytosolic).¹⁰² We were the first to report that the anticarcinogenic effects of triptolide were mediated via a lysosomal mechanism.¹⁰² This shift in fluorescence indicates that lysosomal membrane rupture occurred. Hsp70, a potent survival protein whose depletion results in caspase-independent apoptosis, has been shown to protect several cancer cells against death stimuli by inhibiting LMP.⁴⁴ Another report showed that clioquinol, a metal chelator, targets zinc to lysosomes causing LMP, release of cathepsins, and apoptosis.¹⁰⁴ Several reports indicate that anticancer agents that target lysosomes and induce lysosomal membrane

disruption play a role in the fight against cancer. For example, chloroquine, a lysosomotropic drug, may prove to be such an agent and it has been reported to inhibit cell growth and induce cell death in lung cancer cells,¹⁰⁵ myc-induced model of lymphoma,¹⁰⁶ and has been used in clinical trials as adjuvant therapy for patients with glioblastoma multiforme.¹⁰⁷ However, the antitumor effects of chloroquine are primarily based on its ability to inhibit autophagy.^{108–110} A recent study described how a novel agent, ARN5187, which has similar inhibitory properties on autophagy as chloroquine, is more cytotoxic to breast cancer cells compared to chloroquine treatment.¹¹¹ Several agents that trigger LMP are currently utilized because of their antitumor effects (Table 1). This table is not meant to be an inclusive list of all the currently used lysosomotropic agents and cathepsin inhibitors.

It has been reported that the parvovirus H1 induces a nonapoptotic cell death in glioma cells that is mediated by the release of cathepsins in response to LMP.¹¹² Exposure of immortalized and transformed cells to siramesine, a sigma-2 receptor agonist, in vitro has been demonstrated to result in extensive cell death.^{15,113–116} One study screened a drug library and identified 175 compounds that induced death in cultured colon cancer cells.¹¹⁷ Importantly, over half of the eleven compounds that induced significant cell death in p53-deficient cells triggered LMP and cathepsin-mediated killing of tumor cells.¹¹⁷ There is definitely a dire need for targeted anticancer therapies. To this end, a recent report employed a fluorescent small organic molecule, 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC), that selectively labeled the nuclei of cancer cells, but not normal cells.¹¹⁸ The authors of this study demonstrated that BMVC was retained in the lysosomes and was excluded from the nuclei of normal cells, which could explain the

Table 1 Various lysosomotropic agents and specific lysosomal protease inhibitors that possess anticancer properties

Treatment	Cell type	Protein(s)	Reference
Leelamine	Melanoma	RTK-AKT/STAT/MAPK	124
BMVC	MCF-7, foreskin, and lung fibroblasts	Unknown	118
Geldanamycin	HTB-26, MDA-MB-231, HEK 293T, HL-60, fibroblasts	Hsp90	125
EGCG	HepG2, mouse embryonic fibroblasts	Unknown	126
Triptolide	MCF-7	Cathepsin B	102
Chloroquine	MEF, PC3, U87MG	PI3K-Akt	127
D-e-MAPP	MCF-7	Unknown	128
Symadex (C1311)	HT-29	Acid phosphatase	94
Z-Phe-Gly-NHO-Bz	BT-20, PC3, U373, SQ20B, HELA, DU 145	Cysteine cathepsins	129
α -ketoamide	A2058	Cathepsin S	130
JMV4463	MDA-MB-231, MCF-7, LNCaP, SaOs2, HCT-116	Cathepsin D	131
BPC	K562	Cathepsin B	92
CLIK-148	LJC-1, A375, 26 PMF-15	Cathepsin L	132
Kalpaamruthaa	DMBA mammary gland xenograft	Cathepsin D	133

Abbreviation: BMVC, 3,6-bis(1-methyl-4-vinylpyridinium) carbazole; EGCG, Epigallocatechin Gallate; BPC, Biphosphinic Palladacycle Complex.

observed selective hyperfluorescence of BMVC in cancer cell nuclei.¹¹⁸ These findings are very promising and suggest that it may be possible in the future to exploit differences in subcellular localization for cancer targeting for treatment modalities. Support for this theory is found in the fact that normal cells have acidic lysosomes with a pH range of 4–5, whereas the cytosol is primarily neutral.¹¹⁹ Consequently, weak bases will accumulate in acidic organelles such as lysosomes. Several cancer cells have defects in lysosomal acidification.^{120–122} For instance, the lysosomal pH of HL-60 cells is 6.5, however the cytosolic pH of these cells is neutral.¹²³

Cathepsin inhibitors

Several recent reports have employed an antibody, an antibiotic, and a small molecule as potential therapeutic strategies for the treatment of tumors by specifically inhibiting the activities of various cathepsins^{94,124–126} (several of these agents are summarized in Table 1). In one study, the antitumor activity of Fsn0503h, the first human monoclonal antibody developed against extracellular cathepsin S, was examined.¹²⁷ In that study the authors demonstrated that Fsn0503h does not affect the intracellular activity of cathepsin S and has in vivo antitumor activity in a Colo-205 mouse xenograft model.¹²⁷ Nitroxoline is an antibiotic that possesses antiangiogenic activity.¹²⁸ A recent report has shown that nitroxoline inhibited tumor growth, angiogenesis, and metastasis in vivo in breast cancer and fibrosarcoma models.¹²⁹ L-235 is a cathepsin K inhibitor that has been reported to reduce skeletal breast cancer tumor burden and breast cancer local metastasis in a xenograft model.¹³⁰

Conclusion

With the development of novel, specific fluorescent probes for staining lysosomes¹³¹ in vivo, such as Superior LysoProbes, we should be able to have a better understanding on the exact mechanisms that regulate lysosomal membrane destabilization. Cell lines, such as MCF-7, which lack the apoptosis executioner protein caspase-3¹³² and can undergo lysosomal-mediated cell death,¹⁰² should prove useful in deciphering the role of lysosomes as novel pharmacologic targets in human tumors. The utilization of anticancer agents that stimulate apoptosis via LMP and/or that effectively inhibit the extracellular tumor-promoting activities of cathepsins, should further support the hypothesis that targeting lysosomes is an effective form of cancer treatment. These data suggest that lysosomotropic drugs and specific lysosomal protease inhibitors may prove useful in the treatment of otherwise therapy-resistant human cancers and

further research is warranted to identify such agents. Tumor cells exhibit specific alterations in their lysosomes: increased cathepsin enzymatic activity, modified lysosomal trafficking, and shifts in different endolysosomal populations.³³ We propose that these differences in the lysosomes of cancer cells compared to normal cells can be exploited to sensitize tumors to cell death. The importance of lysosomal-mediated cell death has been underappreciated, yet holds great promise in the fight against chemoresistance because it provides an alternative pathway to kill cancer cells.

Disclosure

The author reports no conflicts of interest in this work.

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