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Mini-review

## Combating apoptosis and multidrug resistant cancers by targeting lysosomes

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### ABSTRACT

Acquired therapy resistance is one of the prime obstacles for successful cancer treatment. Partial resistance is often acquired already during an early face of tumor development when genetic changes causing defects in classical caspase-dependent apoptosis pathway provide transformed cells with a growth advantage by protecting them against various apoptosis inducing stimuli including transforming oncogenes themselves and host immune system. Apoptosis defective cells are further selected during tumor progression and finally by apoptosis inducing treatments. Another form of resistance, multidrug resistance, arises during cancer treatment when cancer cells with effective efflux of cytotoxic agents escape the therapy. Remarkably, induction of lysosomal membrane permeabilization has recently emerged as an effective way to kill apoptosis resistant cancer cells and some lysosome targeting drugs can also re-sensitize multidrug resistant cells to classical chemotherapy. In this review, we highlight recent data on lysosomal cell death pathways and their implications for the future treatment of apoptosis defective and multidrug resistant aggressive tumors.

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### 1. Introduction

Emerging data from model systems based on genetic manipulations have identified lysosomal leakage as an important effector of several evolutionarily conserved stress-induced cell death programs *in vitro* and *in vivo* [1–7]. Massive lysosomal leakage followed by a release of lysosomal hydrolases into the cytosol leads to an uncontrolled necrosis, whereas minor membrane permeabilization can activate either the intrinsic caspase-dependent apoptosis pathway or caspase-independent alternative cell death programs [8–12]. Lysosomotropic detergents, basic compounds that accumulate in acidic lysosomes and disrupt them from within, were considered as putative anti-cancer drugs already over thirty years ago [13]. This approach was, however, discarded due to an assumption that all lysosome-bearing cells, i.e. all cells except for mature

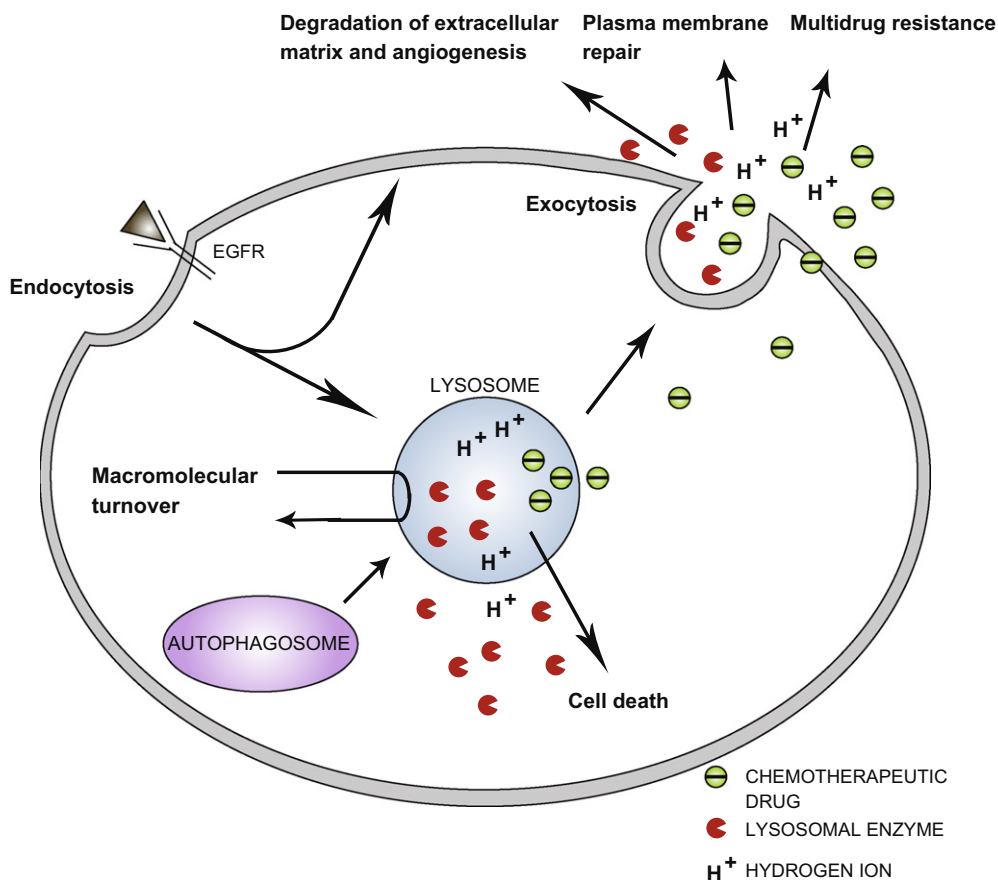
erythrocytes, would be equally vulnerable to the cytotoxic action of such compounds. Accumulating data show, however, that cancer is associated with dramatic lysosomal changes that promote invasive growth and angiogenesis (reviewed in [14,15]) (Fig. 1), and the realization that these changes greatly sensitize cells to lysosomal membrane permeabilization (scope of this manuscript) has initiated a new wave of interest in lysosomes as targets for cancer therapy [6,16,17].

### 2. Lysosomes – more than housekeeping organelles

Christian de Duve and colleagues discovered lysosomes over 50 years ago and described them as cellular acidic organelles surrounded by a single membrane and filled with acid hydrolases [18]. For many years lysosomes were merely considered as ‘boring’ housekeeping organelles that degrade old organelles and macromolecules making them available for metabolic reuse. The degradation is now known to be quite specific in some cases, and lysosomes

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**Fig. 1.** Lysosomal involvement in cancer. Besides degrading and recycling organelles and macromolecules received via autophagy or endocytosis, lysosomes and their contents can serve multiple localization-dependent functions in cancer. For example cathepsins mediate cell death when released to the cytosol upon LMP, and promote invasion and angiogenesis when released to the extracellular space via lysosomal exocytosis. Lysosomal exocytosis is also involved in plasma membrane repair as the source of membrane and in multidrug resistance through exocytosis of drugs sequestered in the lysosomal compartment and the acidification of the extracellular space. EGFR: Epidermal growth factor receptor.

play important roles also in the repair of plasma membrane, removal of pathogens, recycling of cell surface receptors, survival of starved cells and release of lysosomal contents [19–21] (Fig. 1). Lysosomes are highly dynamic organelles and the material to be degraded is brought to the organelle by endocytosis, phagocytosis and autophagy. In order to accomplish the degradation task, lysosomes contain a large number of hydrolases capable of degrading the majority of cellular macromolecules. The best described are the proteases cathepsins that can be divided into three groups, namely cysteine (B, C, H, F, K, L, O, S, V, W and X/Z), aspartate (D and E) and serine (A and G) cathepsins [22]. Other lysosomal hydrolases that have drawn the attention of cancer researchers include sphingomyelin-degrading lipases that regulate the levels of important membrane lipids and cellular second messengers such as ceramide, sphingosine and sphingosine-1-phosphate [23].

Besides the lysosomal hydrolases, the transmembrane proteins of lysosomal membranes are also important for proper lysosomal function. Vacuolar  $H^+$ -ATPase pumps protons into the lysosomes and ensures optimal working conditions for lysosomal hydrolases, most of which function optimally in acidic pH. Lysosome-associated mem-

brane proteins 1 (LAMP-1) and 2 (LAMP-2), lysosome membrane protein 2 (also known as lysosomal integral membrane protein 2) and CD63 (also known as lysosomal integral membrane protein 1) are among the other abundant lysosomal membrane proteins. These and other membrane proteins have been suggested to control the membrane stability, membrane fusion, macromolecule import from the cytosol to the lysosome, transport of degradation products back to the cytosol as well as trafficking of the organelle and its hydrolases [20,24]. The massive glycosylation of luminal domains of LAMP-1, LAMP-2 and lysosome membrane protein 2 forms a 'sugar coat' or glycocalyx inside the lysosomal limiting membrane and protects thereby the membrane against degradation by lysosomal hydrolases.

### 3. Lysosomal cell death

#### 3.1. When apoptosis fails, lysosomes can take over

Apoptosis is a highly sophisticated cell death program that has been acquired by multicellular organisms in order

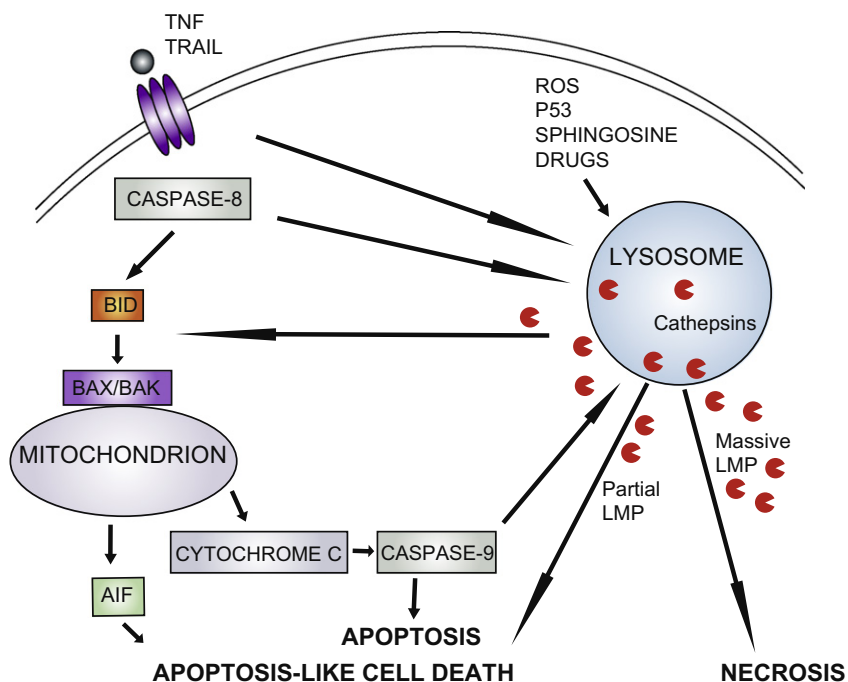
to maintain cellular homeostasis and eliminate unwanted genetically damaged or senescent cells [25]. It is characterized by morphological hallmarks such as cell shrinkage, chromatin condensation, membrane blebbing and nuclear fragmentation eventually leading to the fragmentation of the cell to apoptotic bodies. In mammalian cells, two major molecular pathways can lead to the apoptotic process, the death receptor pathway and the mitochondrial pathway referred to as extrinsic and intrinsic pathways, respectively [26–29]. The first one is initiated at the plasma membrane by the activation of death receptors of the tumor necrosis factor (TNF) family that activate caspase-8, followed by caspase-3 and -7 activation [26,27]. The intrinsic pathway is activated by various stimuli, e.g. growth factor starvation, DNA damage, heat, disruption of cytoskeleton and endoplasmic reticulum stress, and involves mitochondrial outer membrane permeabilization (MOMP), cytochrome c release and subsequent activation of caspase-9 and effector caspases. The pro-apoptotic members of the Bcl-2 family link the various stresses to MOMP and accordingly the anti-apoptotic Bcl-2 proteins effectively block the intrinsic apoptosis pathway [28]. The extrinsic apoptosis pathway is connected to the intrinsic pathway through Bid, a pro-apoptotic Bcl-2 family member, which can be cleaved by caspase-8 to an active form (tBid) capable of inducing MOMP via pro-apoptotic proteins Bax and Bak (Fig. 2).

Apoptosis is by far the best-studied cell death pathway and the development of anti-cancer drugs has mainly aimed at targeting this pathway. It has, however, become

clear that this pathway is frequently defect in cancer cells [30,31]. For example, mutations in the pro-apoptotic proteins Bax or Bak or over-expression of the anti-apoptotic proteins Bcl-2, Bcl-xL and Mcl-1 are relatively common in cancers. Therefore, attention has been driven towards other cell death pathways that circumvent the signaling events of classical apoptosis. This is exactly what the lysosomal cell death is able to do, and for that reason this cell death pathway has gained increasing attention over the last years.

### 3.2. Lysosomal membrane permeabilization

Lysosomal membrane permeabilization (LMP) resulting in the release of lysosomal hydrolases into the cytosol is the major hallmark of lysosomal cell death pathways. How LMP is exactly carried out is still debated and there may be a number of correct answers to this question depending on the inducing stimuli and cell type. Whereas lysosomotropic detergents are likely to permeabilize the membrane directly, the growing list of other inducers of LMP has no obvious common nominator (Table 1). TNF is the best-studied inducer of LMP, and many of the reported mediators of LMP, i.e. cysteine cathepsins, caspase-8, caspase-9, Bid, sphingosine, reactive oxygen species and p53, have been associated with TNF-induced LMP [32–35] (Fig. 2). Thus, we present the literature on the molecular basis of LMP with focus on TNF signaling. It should, however, be noted here that induction of LMP by TNF may not be the optimal strategy to treat apoptosis resistant



**Fig. 2.** Lysosomal cell death pathways. Numerous treatments trigger LMP either directly or indirectly resulting in the release of lysosomal content into the cytosol. Here, lysosomal hydrolases, especially cathepsins, can mediate cell death either in a mitochondrion-independent manner or through cleavage-mediated activation of pro-apoptotic Bcl-2 family members and subsequent release of apoptogenic factors (e.g. cytochrome c and AIF) from the mitochondria (please refer to the text for details).

**Table 1**

Lysosomal cell death induction by established and putative cancer treatments.

TREATMENT	CELL TYPE <sup>a</sup>	MEDIATOR <sup>b</sup>	REFERENCE
<b>Antibiotics:</b>			
Cipro- & norfloxacin	Cervix carcinoma	Unknown	[49]
Nanaomycin & Siomycin A	Colon carcinoma	CathD	[87]
<b>Antibodies to:</b>			
Fas	Cervix carcinoma and leukemia	Cath D	[88]
CD20 (Tositumomab)	Lymphoma	Cath B	[60]
HLA-DR (L243)	Lymphoma	Cath B	[60]
<b>Cytokines:</b>			
Interferon $\gamma$	Cervix carcinoma and leukemia	Cath D	[88]
TNF	Breast and cervix carcinoma, murine hepatocytes and fibroblasts	Cath B/L	[3,6,32,89]
TRAIL	Cervix carcinoma and leukemia, murine fibrosarcoma	Cath D	[70,88,90,91]
	Leukemia	Cath D	[92]
	Hepatocellular and squamous cell carcinoma, murine fibrosarcoma	Cath B	[3,91,93]
<b>DNA-damaging drugs:</b>			
Camptothecin	Leukemia and lymphoma	Cath B	[94]
Cisplatin	Murine embryonic fibroblasts	Cath B, L	[16]
Etoposide	MEFs	Cath B, L	[16]
	Eukemia	Cath D	[91]
<b>Kinase inhibitors:</b>			
Imatinib (Bcr-Abl)	Leukemia, CD34+ hematopoietic cells	Cath B	[72]
Bcr-Abl siRNA	Leukemia	Cath B	[72]
Staurosporine (Protein kinase C)	Activated T-lymphocytes and fibroblasts	Cath D	[71,95]
<b>Lysosomotropic compounds:</b>			
Hydroxychloroquine	Cervix carcinoma	Cath B	[96]
BAMLET	Breast carcinoma	Cath B, L	[38]
Siramesine	Breast carcinoma	Cath B, L	[44]
LeuLeuOMe	Cervix carcinoma	Cath B, L	[97]
Sphingosine	Leukemia	Cath B, L, D	[39]
<b>Microtubule-targeting drugs:</b>			
Discodermolide	Non small cell lung carcinoma	Cath B	[58]
Docetaxel	Prostate carcinoma	Cath B	[65]
Epithilone B	Non small cell lung carcinoma	Cath B	[58]
Paclitaxel	Non small cell lung carcinoma	Cath B	[58]
Vincristine	Cervix carcinoma	Cath L	[59]
<b>Natural products:</b>			
$\Delta^9$ -tetrahydrocannabinol	Rat primary cortical neurons	Cath D	[55]
Resveratrol	Cervix carcinoma	Cath L	[68]
Helenalin	Colon carcinoma	CathD	[87]
<b>Proteasome Inhibitors:</b>			
PS-341 (Bortezomib)	Pancreas carcinoma	CathB	[48]
NSC687852, NSC638646, MG-132, Disulfiram & PDTC	Breast carcinoma	Cath D	[98]
<b>V-ATPase inhibitors:</b>			
Omeprazole	Leukemia, lymphoma	Cath B, L	[47,99]
Bafylomycin A1	Gastric carcinoma	Cath D	[100]
<b>Others:</b>			
Retinoid (CD437)	Leukemia	Cath D	[101]
Embelin (XIAP inhibitor)	Colon Carcinoma	Cath B	[56]
Photodynamic therapy	Murine hepatoma	Unknown	[51]
Parvovirus	Glioma	Cath B, L	[42]
Hsp70 antisense	Breast carcinoma	Cath B, L	[61]
Hsp70-2 & LEDGF siRNAs	Brest carcinoma	Cath B, L	[43]

<sup>a</sup> Refers to human cells if not otherwise indicated.<sup>b</sup> Suggested mediator downstream of LMP, whose genetic or pharmacological inhibition inhibits or delays cytotoxicity.

cancers because contrary to many other LMP inducers TNF-induced LMP usually depends on caspase activation with an exception of a few cell lines where caspase inhibition can even enhance LMP [3,36] (Fig. 2). Instead, compounds directly targeting the lysosomes (e.g. siramesine and BAMLET) may prove more successful because they do not de-

pend on specific signaling pathways for the induction of LMP and thereby circumvent existing defects in apoptosis and other signaling pathways [37,38].

TNF fails to induce LMP in cathepsin B deficient hepatocytes indicating that cathepsin B (or lysosomal cysteine cathepsin activity in general) is essential for TNF-induced

LMP [32]. A more general role of cathepsins in lysosomal membrane stability is supported by data showing that transformation-associated increase in lysosomal cysteine cathepsin activity reduces the half-life of LAMP-1 and LAMP-2 and thereby destabilizes lysosomal membranes and sensitizes cells to various LMP-inducing stimuli [16]. Additionally, cathepsins can regulate the level of sphingosine, a potent detergent that induces a dose-dependent LMP in Jurkat T cells and murine hepatocytes [32,39]. TNF induces an increase in sphingosine levels prior to LMP and also sphingosine fails to induce LMP in cathepsin B deficient murine hepatocytes [32]. Thus, cathepsin B activity (e.g. via the degradation of LAMP-1 and LAMP-2) may be necessary for sphingosine to reach the lipid layer of the lysosomal limiting membrane, or alternatively cathepsin B could increase the half-life of sphingosine. The latter hypothesis is supported by data showing that TNF can activate cathepsin B-mediated degradation of sphingosine kinase-1 that converts pro-apoptotic sphingosine to anti-apoptotic sphingosine-1-phosphate [40]. Thus, high levels of sphingosine kinase-1 in cathepsin B deficient cells may rapidly convert sphingosine to sphingosine-1-phosphate. Even though sphingosine is an attractive candidate as a mediator of TNF-induced LMP, the direct evidence supporting this connection is still lacking.

Even though LMP enhances TNF-induced caspase activation, caspase-9 is essential for TNF-induced LMP in most cells. Murine caspase-9 can be cleaved and activated directly by caspase-8 allowing a mitochondrion-independent LMP induction. Human caspase-9 cannot be cleaved by caspase-8, and accordingly in human cancer cells TNF-induced LMP depends on MOMP, cytochrome c release and apoptosis-mediated activation of caspase-9 [3,34]. Thus, caspase-9 may function as a more general link between the intrinsic apoptosis pathway and LMP. Additionally, Gores and co-workers have shown that caspase-8-mediated activating cleavage of Bid links caspase activation to LMP in murine hepatocytes [33]. LMP occurs, however, normally in TNF-treated Bid deficient murine embryonic fibroblasts and in Bid-depleted cholangiocarcinoma cells treated with TNF-related apoptosis inducing ligand (TRAIL) [34,41]. TNF-induced LMP in murine embryonic fibroblasts is also unaffected by combined lack of Bax and Bad, whereas TRAIL-induced LMP in cholangiocarcinoma cells is partially dependent on Bim and Bax. Thus, the role of pro-apoptotic Bcl-2 proteins in the induction of LMP is rather controversial. One explanation for the controversial data can be the ample crosstalk between mitochondrial Bcl-2-regulated apoptosis pathway and LMP, which makes it difficult to differentiate between lysosomal and mitochondrial effects of Bcl-2 proteins on LMP induction in apoptosis competent cells. Finally, it should be noted that the failure of Bcl-2 to suppress the cell death is one of the major hallmarks of most lysosomal cell death models in apoptosis defective cancer cells strongly suggesting that LMP can occur independent of Bax and Bak [34,38,42–45].

TNF-induced caspase-independent cell death has been linked to an increase in reactive oxygen species (ROS) [46]. Many other known inducers of LMP (e.g. bortezomib, omeprazole, norfloxacin and siramesine) also promote production of ROS [44,47–49], and direct treatment of cells

with oxidants or photo-oxidation trigger LMP [50,51]. As a site of intracellular degradation of organelles and long-lived proteins, lysosomes accumulate labile forms of iron released from metalloproteins. In the presence of ROS, gathered iron can catalyze Fenton reactions that create highly reactive hydroxyl radicals that are likely to destabilize the lysosomal membrane. The ability of the iron chelator deferoxamine to inhibit oxidant-induced lysosomal damage strongly supports the role of Fenton chemistry in oxidant-induced LMP, but the role of ROS and iron in LMP induced by other stimuli remains speculative. In addition to direct effects of lysosomal ROS in LMP, cathepsin B released to cytosol can enhance ROS formation in mitochondria and activate phospholipase A2, which is capable of inducing LMP thereby creating a positive feedback loop [52,53].

Finally, LMP induced by TNF and several other compounds (e.g. embelin and  $\Delta^9$ -tetrahydrocannabinol) has been suggested to depend on p53, and ectopic expression of p53 is sufficient to trigger LMP [35,54–56]. The recruitment of p53 to the lysosomal membrane has been shown to depend on the association of phosphorylated p53 (Ser 15/18) with lysosome-associated apoptosis-inducing protein containing the plekstrin homology and FYVE domains (LAPF), a protein that when overexpressed can induce a cathepsin D dependent apoptosis in murine fibrosarcoma cells [35,57]. Thus, p53 may also link other stimuli that induce p53 phosphorylation, e.g. DNA damaging agents and metabolic stress, to LMP and lysosomal cell death pathway.

Another strategy to induce LMP is to interfere with lysosomal trafficking by disturbing microtubule or actin dynamics. Microtubule-stabilizing (e.g. paclitaxel, epothilone B and discodermolide) and destabilizing (vincristine) agents induce early LMP [58,59], and LMP induced by so called type II monoclonal antibodies against B cell surface antigens CD20 (tositumomab) and HLA-DR (L243) in B cell lymphomas depends on actin reorganization [60]. In both cases dramatic lysosomal swelling and an increase in lysosomal cysteine cathepsin activity precedes LMP. The mechanism by which disturbance of lysosomal trafficking leads to LMP remains, however, obscure. One possibility is that lysosomal stability depends on continuous trafficking-dependent fusion events with other vesicles or plasma membrane.

In addition to the existing drugs, LMP can also be induced by genetic or pharmacological inhibition of proteins that stabilize the lysosomal membrane, e.g. the major stress-inducible member of the heat shock protein 70 (Hsp70) family (here referred to simply as Hsp70, also known as Hsp72, Hsp70-1 and HSPA1) and the highly related cancer-associated Hsp70-2 (also known as HSPA2) [43,51,61,62]. Hsp70 is a molecular chaperone that promotes cell survival by inhibiting lysosomal membrane permeabilization. In cancer cells and upon other stresses Hsp70 translocates to lysosomal lumen, where it binds to an anionic phospholipid bis(monoacylglycero)phosphate (BMP), an essential co-factor for lysosomal sphingomyelin metabolism, and facilitates acid sphingomyelinase (ASM) activity [63]. Inhibition of Hsp70 – BMP interaction by a single point mutation in Hsp70 or antibodies against BMP as well as pharmacological and genetic inhibition of

ASM inhibits the Hsp70-mediated stabilization of lysosomes indicating that ASM activity is essential for lysosomal stability [63]. In spite of the great sequence homology between Hsp70 and Hsp70-2, the mechanisms by which they stabilize lysosomes are very different. Hsp70-2 does not localize to lysosomes and even when forced to this location it fails to stabilize them [63]. Instead, it enhances the expression of a cancer-associated protein, lens epithelium-derived growth factor (p75/LEDGF) that effectively inhibits LMP by an as yet unknown mechanism [43,64,65].

### 3.3. LMP as a trigger of the intrinsic apoptosis pathway

Even though cell death pathways are often studied as separate entities, it is important to note that an ample crosstalk between the different death programs is rather a rule than an exception [11] (Table 1). Accordingly, several mechanisms connect the lysosomal cell death pathway to other death pathways. As discussed above, caspase 9 can link the intrinsic apoptosis pathway to LMP, which can then serve as a back-up cell death mechanism if the effector caspase activation is defective in the target cell. Vice versa, LMP leads to MOMP, release of cytochrome c and caspase activation in apoptosis competent cells [49]. Thus, LMP-initiated and cathepsin-dependent cell death often leads to apoptotic morphology and is classified as classic caspase-dependent apoptosis.

Accumulating data support the role of cytosolic cathepsins, especially cysteine cathepsins B and L and aspartate cathepsin D, as effectors of LMP-initiated cell death pathways including LMP-induced apoptosis. Even though cathepsins function optimally at acidic pH, they do retain some activity at the neutral pH of the cytosol [66]. On the other hand, the cytosol can also become slightly more acidic following the release of lysosomal hydrogen ions to the cytosol thereby prolonging the activity of released cathepsins. The first evidence for the ability of cytosolic cathepsins to trigger cell death came from Karin Roberg's elegant studies showing that cathepsin D injected into the cytosol is sufficient to trigger MOMP, cytochrome c release and apoptosis in human fibroblasts [67]. The evidence for cathepsins B and L as cytosolic effectors of cell death is less direct and mainly based on the ability of cytosolic protease inhibitors (cystatins and serpins) to block LMP-induced cell death [3,5,42,68], and the identification of cytosolic cysteine cathepsin substrates as possible mediators of cell death. One such substrate is Bid, a pro-apoptotic BH3-only protein of the Bcl-2 family that can be processed and activated by several cysteine cathepsins (B, H, L, S, and K) at cytosolic pH. Furthermore, degradation of anti-apoptotic Bcl-2 family members by lysosomal cathepsins can synergize with cathepsin-mediated activation of Bid to trigger a mitochondrial pathway to apoptosis [69]. Even though cathepsin D cannot cleave Bid in neutral pH *in vitro*, it has been suggested to do so in TNF-treated cells [70]. Bid is, however, not a universal mediator of cathepsin D-dependant MOMP because in staurosporine-treated activated T lymphocytes, cathepsin D activates Bax in a Bid-independent manner [71]. Also in fibroblasts treated with ciprofloxacin or norfloxacin, LMP triggers

MOMP through Bid-independent activation of Bak and Bax. In this model system deficiency of cathepsin B, L or D fails to inhibit MOMP suggesting that the cytotoxicity is mediated by a combined effect of several cathepsins or by other effectors [49]. It should be noted that very little effort has been invested to the investigation of the role of other lysosomal hydrolases (e.g. phospholipases) in cell death even though they at least theoretically could promote MOMP once released to the cytosol [53].

### 3.4. LMP as a trigger of non-apoptotic cell death pathways

Importantly, accumulating data show that in addition to activating the intrinsic apoptosis pathway, LMP can trigger caspase-independent cell death with either apoptosis-like or necrotic morphology [9,11]. Existing and emerging anti-cancer drugs shown to trigger such a cell death pathways include microtubule-disturbing agents, kinase inhibitors, antibodies against B-cell antigens and cancer selective lysosomotropic compounds [38,44,58–60,72]. It is as yet unclear whether the ability of LMP inducers to trigger caspase-independent cell death depends on the extent of the LMP, cell type studied, additional effects of the treatment used or combination of all factors. Studies employing increasing concentrations of sphingosine in apoptosis competent cells strongly suggest that a small leakage of lysosomal hydrolases triggers the intrinsic apoptosis pathway whereas a large leakage results in caspase-independent cell death [39]. On the other hand, the cell type studied has a great impact on the cell death program observed because caspase-independent pathways are often slower than apoptosis and thus become evident only in apoptosis incompetent cells. Finally, the treatment may also have other effects that define the outcome. For example, parallel induction of LMP by ciprofloxacin and norfloxacin combined with UV radiation in murine embryonic fibroblasts leads to Bcl-2 sensitive and insensitive cell death, respectively [49].

Whereas the necrosis induced by a massive LMP can be explained by an uncontrolled disintegration of the cell by the leaked cathepsins and other hydrolases, the molecular mechanisms involved in LMP-induced caspase-independent apoptosis-like cell death are less clear. The role of cysteine cathepsins as mediators of more controlled cell demise is supported by the ability of pharmacological and genetic inhibition of cathepsin B to rescue WEHI-S murine fibrosarcoma cells from apoptosis triggered by TNF or TRAIL [3]. Furthermore, inhibition of cathepsin B activity in cells treated with microtubule stabilizing drugs prevents the appearance of multinucleated cells, an early characteristic of cell death induced by microtubule-disturbing agents, pointing to a central and proximal role for cathepsin B [58]. And finally, cathepsin B can induce nuclear apoptosis in isolated nuclei [73]. The special role of cathepsin B in the cellular models described above may be due to the high expression of this cysteine cathepsin in many cancer cells rather than its specific ability to promote apoptosis-like cell death. Another way to achieve caspase-independent apoptosis-like nuclear morphology has been described in LMP models where cathepsin D plays a prominent role. Upon staurosporine-induced LMP in acti-

vated T-cells and TNF-induced LMP in L929 murine fibrosarcoma cells, the leaked cathepsin D induces a selective mitochondrial release of apoptosis-inducing factor (AIF), a protein capable of inducing caspase-independent cell death characterized by partial DNA fragmentation and chromatin condensation [57,71].

In complete contrast to the role of cathepsins as mediators of cell death, it should be mentioned that inhibitors of both cathepsin B and D are able to induce a caspase-dependent cell death in aggressive neuroblastoma cell lines [74]. Since cathepsins are essential for autophagic flux, the death may be a result of the inhibition of this cytoprotective metabolic function of lysosomes.

#### 4. Lysosomes and multidrug resistance

The term multidrug resistance (MDR) defines a phenotype where cells are resistant to multiple drugs with no obvious structural resemblance and with different molecular targets. The phenomenon is typically connected to over-expression of P-glycoproteins that function as ATP-dependent drug efflux pumps in the plasma membrane. Other factors have also been suggested to contribute to MDR and many of them are connected to the endo-lysosomal compartment. The role of the endo-lysosomal compartment in MDR was initially suggested because the distribution of chemotherapeutic drugs changes from cytosolic to endo-lysosomal upon development of MDR. Several chemotherapeutic drugs are weak bases, and many MDR tumors and cell lines have increased the pH gradient between the cytosol and lysosomes resulting in the accumulation of the basic drugs in lysosomes by simple diffusion [75]. Moreover, MDR cells possess an increased rate of endo- and exocytosis, which possibly increases the efflux of drugs accumulated in the endo-lysosomal compartment [76]. Finally, MDR cells have increased number, volume and membrane area of endosomal system and thereby they comprise a great capacity for sequestering large amounts of chemotherapeutic drugs [77].

Recently, attention has been driven to pH gradients between extra- and intracellular spaces of tumor cells as mediators of MDR. The extracellular milieu of solid tumors is significantly more acidic than that of normal tissues, and could thus impair the uptake of weak bases through the plasma membrane and reduce their effect on tumors [78,79]. This might be mediated via mechanisms like decreased tumor reperfusion and metabolic abnormalities associated with transformation [80], but could as well be linked to lysosomes, because the increased rate of lysosomal exocytosis can acidify tumor microenvironment (Fig. 1).

Drugs that are able to revert MDR are often lysosomotropic compounds that inhibit lysosomal function by increasing lysosomal pH via cationic neutralization, inhibition of vacuolar H<sup>+</sup>-ATPase or destabilization of lysosomal membranes [80,81]. Neutralized lysosomes can neither accumulate basic drugs nor exocytose H<sup>+</sup>-ions to the exterior, thus favoring retention of chemotherapeutic drugs in the cell. Some 'lysosomal inhibitors' such as chloroquine are additionally able to directly inhibit the P-glycopro-

tein-mediated efflux of the drugs by competing for the binding to P-glycoprotein [82,83]). Vice versa, in addition to its efflux function in the plasma membrane, P-glycoprotein can also enhance lysosomal exocytosis of lysosomal hydrolases together with sequestered drugs [84].

A number of drugs that can revert MDR, e.g. chloroquine and desipramine, also inhibit the activity of the lysosomal ASM that hydrolyses sphingomyelin to ceramide [85]. This is interesting because inhibition of ASM also destabilizes the lysosomal membrane and thus renders cells particularly vulnerable to lysosome targeting drugs. Despite several attempts, inhibitors of MDR have not yet been successful in clinics. The main problems encountered include the toxicity of MDR inhibitors themselves as well as the uncontrollable increase in the side-effects of the anti-cancer drug [86]. Taking into account the new knowledge on lysosomal cell death, it is tempting to speculate that targeting LMP in cancer cells with drugs that reduce ASM activity might both induce cell death independent of classical apoptotic pathway and inhibit the process of MDR progression, or even revert an already existing MDR.

#### 5. Concluding remarks

The lysosomal compartment is dramatically altered in cancer cells presenting increased volume, elevated expression and activity of lysosomal enzymes as well as changed trafficking [17]. These changes correlate with aggressiveness of tumors and might also take part in the development of MDR. Therefore, drugs that induce lysosomal cell death pathway independently of caspases could be particularly efficient in cancer treatment. One argument is that the enlarged lysosomal system in cancer cells render them more vulnerable to such drugs compared to normal cells thus reducing the number of side effects. Another argument is that induction of LMP theoretically would reduce the capability of chemotherapeutic drugs to accumulate in lysosomes and be more efficient in the cytosol and the nucleus. Thus, cancer treatments including lysosome targeting drugs hold great promise for being successful in circumventing both apoptosis and multidrug resistance.

#### 6. Conflicts of interest

None declared.

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