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Review

Lysosomal involvement in cell death and cancer

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ABSTRACT

Lysosomes, with their arsenal of degradative enzymes are increasingly becoming an area of interest in the field of oncology. The changes induced in this compartment upon transformation are numerous and whereas most are viewed as pro-oncogenic the same processes also render cancer cells susceptible to lysosomal death pathways. This review will provide an overview of the pro- and anti-oncogenic potential of this compartment and how these might be exploited for cancer therapy, with special focus on lysosomal death pathways.

1. Introduction

Since the discovery of lysosomes by de Duve in 1955[1] this organelle has been mainly viewed as a final destination for endocytic cargo and macromolecules destined for breakdown. This view of the lysosomes as, at best, a garbage disposal unit, and at worst, an unspecific "suicide bag" has changed dramatically due to recent discoveries that provide evidence for numerous more specific tasks for lysosomes and their contents.

As the main compartment for intracellular degradation and subsequent recycling of cellular constituents, the lysosomes receive both hetero- and autophagic cargo, which in the degradative lumen of this organelle find their final destination. The degradation is carried out by a number of acid hydrolases (phosphatases, nucleases, glycosidases, proteases, peptidases, sulfatases, lipases, etc) capable of digesting all major cellular macromolecules [2]. The best-studied lysosomal hydrolases are the cathepsin proteases which can be divided into three sub-groups according to their active site amino acid, i.e. cysteine (B, C, H, F, K, L, O, S, V, W and X/Z), aspartate (D and E) and serine (G) cathepsins [3].

Until recently the function of lysosomes and their cathepsins was thought to be limited to intralysosomal protein-turnover, and the degradation of the extracellular matrix once secreted. However, during the past few years many of the cathepsins have been accredited with more specific functions including roles in bone remodeling, antigen presentation, epidermal homeostasis, prohormone processing, maintenance of the central nervous system in mice, angiogenesis, cell death and cancer cell invasion [4–8]. Importantly, cancer cells show transformation-induced changes of the lysosomal compartment which have pro-oncogenic effects when lysosomal hydrolases participate in tumor growth, migration, invasion and angiogenesis [9] (Fig. 1). Simultaneously, however, the very same changes in the lysosomal compartment may sensitize cells to the lysosomal death pathway, hereby allowing cell death to occur even in cancer cells with multiple defects in the classical apoptosis signaling pathways [10].

This review will seek to offer a detailed view of how the pro- and anti-oncogenic potential of this degradative compartment can be exploited to the benefit of the cancer patient, with emphasis on lysosomal induced cell death.

2. Lysosomes and cancer cell death

Regulation of overall cell number as well as the amount of cells constituting the different tissues along with the need for a mechanism of eliminating unwanted cells is of fundamental importance in multicellular organisms [11]. Apoptosis is the primary means to this end, endowing the multicellular organism with the potential to rid itself of unwanted cells without the leakage of cellular constituents, thus avoiding the inflammation associated with necrosis, the conceptual counterpart to programmed cell death. A recurrent theme in the development of cancers is the development of defects in the complex pathways controlling programmed cell death [10]. Cancer cells often harbor mutations in pro-apoptotic proteins (e.g. Bax, Apaf-1 and p53) and can also rely on the overexpression of anti-apoptotic proteins (e.g. Bcl-2, Bcl-xL, Akt/PKB and inhibitors of apoptotic proteins) as a means to protect them from cell death. As such, cancer cells have a number of opportunities to block classical apoptotic pathways and interfere with efficient caspase activation [10]. Fortunately several lines of evidence suggest that although

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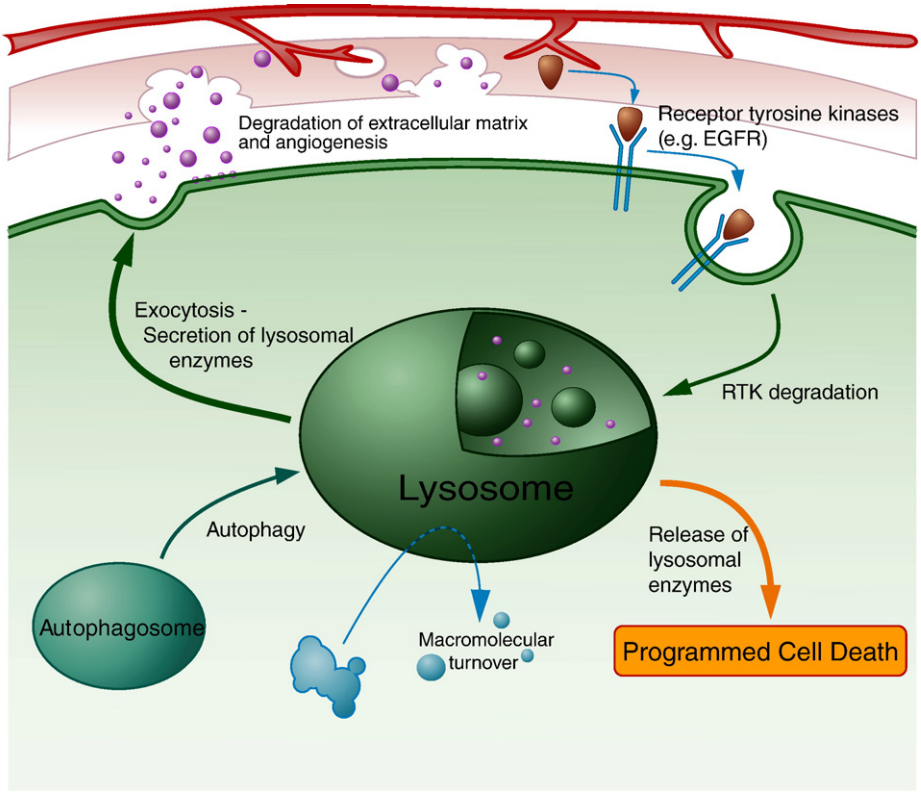

Fig. 1. Lysosomal involvement in cancer. Lysosomes and their enzymes serve multiple roles in cancer depending on the context. An example is the case of release of cysteine cathepsins; if released intracellularly they can contribute to the demise of the cancer cell; if released extracellularly they can act pro-oncogenic in breaking down the extracellular matrix, stimulating angiogenesis and migration. Other roles for lysosomes in cancer include their role as a degradative compartment in the turnover of macromolecules; downregulation of signaling from receptor tyrosine kinases as e.g. the EGFR as well as being the final station in the autophagic pathway. RTK: Receptor tyrosine kinase, EGFR: Epidermal growth factor receptor.

cancer cells may have a block in their normal apoptotic programs, cell death can still occur through the release of lysosomal enzymes. Importantly, the lysosomal cell death pathway can be efficiently triggered by many conventional chemotherapeutic regimens (Table 1 and references herein).

The appreciation of a regulated lysosomal involvement in cell death began within the past decade where the exclusive role of caspases as the executioners of cell death was challenged [12–15].

As newly developed caspase-specific pharmacological inhibitors as well as inactivation of caspase-pathways by different factors [10,16,17] did not always stop the progression towards death, they revealed, or even enhanced, a subset of underlying caspase-independent death programs. These programs include death-receptor initiated pathways [10,18,19] as well as pathways elicited by cancer drugs, growth-factor deprivation, staurosporine, Bax-related proteins and the depletion of Hsp70[16,20–22]. The morphological features of these caspase-independent death programs are often reminiscent of the ones observed for classical apoptosis, and experimental support for a role for other proteases such as cathepsins, calpains and serine proteases as essential cofactors either upstream or downstream of caspases is rapidly growing [14,23–28]. The argument is strengthened by the findings that many non-caspase proteases are able to cleave at least some of the classic caspase substrates, which might explain some of the similarities observed between the caspase-dependent and -independent death programs [14,27,29,30].

The discovery of lysosomal cell death pathways may have been additionally delayed, because the lysosomal ultrastructure appears intact in apoptotic cells analyzed by electron microscopy [31]. Thus, the lysosomal rupture has until recently been considered as an all-or-nothing switch during late stages of uncontrolled necrotic cell death and tissue autolysis [32]. However, newer studies have revealed that lysosomes with normal ultrastructure may have leaked part of their enzymes, and that partial lysosomal membrane permeabilization (LMP) not only occurs early in many death paradigms, but can in fact trigger apoptosis and apoptosis-like cell death [8,27].

Although one can argue the relevance of such lysosomal death programs, as they are masked by the efficacy of the caspases, evidence is gathering for an evolutionarily conserved role for lysosomal cathepsin proteases in cell death programs initiated as a response to various stimuli such as death receptors of the tumor necrosis factor receptor family, hypoxia, oxidative stress, osmotic stress, heat and anti-cancer drugs [15,31,33–35].

2.1. Evidence for lysosomes as cell death initiators

Evidence for the potential of lysosomes as programmed cell death initiators come from studies with various compounds that directly target the integrity of the lysosomal membranes. These have convincingly proven that moderate lysosomal permeabilization can result in programmed cell death [8,36–40]. A quantitative relationship between the amount of lysosomal rupture and the mode of cell death has been suggested to explain the widely different morphological outcomes following LMP [41]. According to this model, low stress intensities trigger a limited release of lysosomal contents to the cytoplasm followed by apoptosis or apoptosis-like cell death, while high intensity stresses lead to a generalized lysosomal rupture and rapid cellular necrosis. Accordingly, low concentrations of sphingosine, an acid ceramidase-generated metabolite of ceramide with detergent-like properties at low pH, induces partial LMP and caspase-mediated apoptosis, whereas higher concentrations result in massive LMP and caspase-independent necrotic cell death. In this model, the death triggered by partial LMP can be inhibited by pharmacological

Table 1
Lysosomal enzymes involved in cancer cell death

Lysosomal enzyme	Cell type	Stimulus	Reference
Cathepsin B and L	Immortalized and transformed murine embryonic fibroblasts	TNF-α	[32]
	MCF-7 human breast cancer	Siramesine	[94]
	HeLa human cervix carcinoma, U-2-OS human osteosarcoma, MCF-7	LEDGF RNAi	[22]
	MCF-7, MDA-MB-468 human breast cancer	Hsp70 antisense	[91]
	WEHI-S mouse fibrosarcoma cells, ME-180 human cervix carcinoma	TNF-α	[91]
	HeLa, MCF-7	Vincristine, vincristine + siramesine	[40]
	U937 human leukemia cells and Namalwa B human lymphoma cells	Camptothecin	[96]
	Human non-small cell lung cancer	Microtubule stabilizing agents	[11]
	MCF-7	TNF-α	[81]
	WEHI-S, ME-180	TNF-α	[35]
	Rat hepatoma cell line	Bile salt	[35]
Cathepsin D	U937	Etoposide, TNF-α, TRAIL	[29]
	HeLa	TNF-α, IFN-γ, anti-Fas	[25]
	HL-60 human leukemia	Synthetic retinoid CD437	[126]
	Jurkat human leukemia T cells	Sphingosine	[65]
	Murine lymphoma cell line	Induction of wild-type p53	[127]
Acid sphingomyelinase	Human glioma cells	Anti-Fas	[107]
	Human glioma cells	γ-radiation	[47]
	HT-29 Human colorectal cancer	TNF-α	[21]
Acid ceramidase	Ewings sarcoma cells	Fenretinide	[6]
	Human glioma cells	γ-radiation	[47]
Not identified	HeLa	L-leucyl-l-leucine methyl ester	[20]
	1c1c7 murine hepatoma	Photodynamic therapy	[16]
	HeLa	Cipro-/Norfloxacin + UV light	[10]
	Jurkat human leukemia T cells	α-tocopherol succinate	[89]
	Jurkat human leukemia T cells	Anti-Fas, oxidative stress, growth factor starvation	[15]
	Human glioblastoma and gastric cancer	Oncogenic Ras expression	[19]

inhibitors of cysteine and aspartate cathepsins, and the increase in the cytosolic cathepsin activity precedes the activation of caspases and mitochondrial membrane potential changes suggesting a direct role for cytosolic cathepsins in the death process. Importantly, the role of LMP and cathepsins in cell death is not limited to the experimental models employing direct lysosomal disrupters. LMP also participates in the execution of cell death in response to a wide variety of classic apoptotic stimuli, such as activation of death receptors of tumor necrosis factor (TNF) receptor family [23,26,42,43], interleukin-1[44], p53 activation [45], growth factor starvation [42], microtubule stabilizing agents [46], etoposide [10,40], sigma-2 receptor activation [47], synthetic retinoid CD437[48], B cell receptor activation [49,50], staurosporine [51], osmotic stress [33], as well as small molecules identified in a screen for novel anti-cancer drugs that induce p53 independent apoptosis [52].

Interestingly, studies employing immortalized murine embryonic fibroblasts (MEFs) from mice deficient for individual cathepsins have clearly revealed that different cathepsins are crucial components in cell death programmes, although their mutual importance varies depending on the stimulus triggering LMP [53]. Immortalized MEFs from cathepsin B and L deficient mice, but not from cathepsin D deficient mice, are e.g. highly resistant to TNF, whereas the opposite picture emerges when the cells are treated with staurosporine. Extensive studies on TNF-induced cell death pathways have further revealed that the role of individual cathepsins in cell death depends on the cell type studied as exemplified by cathepsin D depletion which effectively protects HeLa cervix cancer cells against TNF- and cisplatin-induced cytotoxicity [25,54]. This difference does not appear to be due to general differences between human and murine cells, because cathepsin B alone or together with other cysteine cathepsins is also crucial for the effective TNF-induced killing in human cervix (ME-180) and breast (MCF-7) cancer cell lines [23]. The explanation for this diversity is as yet unknown, but varying expression levels of individual cathepsins and their inhibitors in different cell lines could play a role. Accordingly, the varying ability of different death stimuli to regulate the expression levels of individual cathepsins or their inhibitors could explain the difference in response to different stimuli. For example, adriamycin and etoposide are known to enhance the expression of cathepsin D via the activation of p53[55]. Alternatively, other signaling pathways induced by various stimuli may co-operate with specific cathepsins.

2.2. Signaling to lysosomal membrane permeabilization

Lysosomal membrane permeabilization followed by the release of lysosomal contents, especially cathepsins, to the cytosol is considered to be the key activation step of the lysosomal death pathway. However, aside from the direct membrane-disruptive stimuli described above, the signaling pathways leading to LMP are only beginning to emerge (Fig. 2).

One of the best-studied mechanisms is the signaling from the TNF-receptor 1 although the clarification of this signaling pathway to LMP has been greatly complicated by widely different responses in different target cells.

In summary, TNF can either induce caspase-dependent or -independent permeabilization of lysosomal membranes depending on cellular context [23,56,57]. In addition, the TNF-related ligands FasL, TRAIL and TWEAK have also all been associated with caspase-independent cell death [18,19,23,43,56,58]. Pharmacological and genetic studies indicate that the caspase-mediated pathway leading from TNF to LMP is dependent on caspases-8 and -9, although activation of caspase-9 differs widely between human and murine cells [23,59–61]. The link between caspases and LMP is as yet unknown, and although TNF-induced caspase-8-mediated cleavage of Bid has been suggested to contribute to LMP, these findings could not be verified by TNF-induced LMP in Bid-deficient immortalized MEFs [59,61]. Bid has furthermore been suggested to be a target for cathepsins in lysosomal death pathways implicating Bid downstream, rather than upstream, of the LMP, although this role for Bid has been questioned by work done in a Bid- and cystatin B-deficient mouse model of progressive myoclonus epilepsy [36,62].

TNF also stimulates sphingomyelin breakdown to phosphorylcholine and ceramide by activating neutral sphingomyelinase (SMase) at the plasma membrane and acid SMase (aSMase) in the lysosomal compartment [63]. Both events have been implicated in TNF-induced cell death pathways, but so far only neutral SMase has been connected

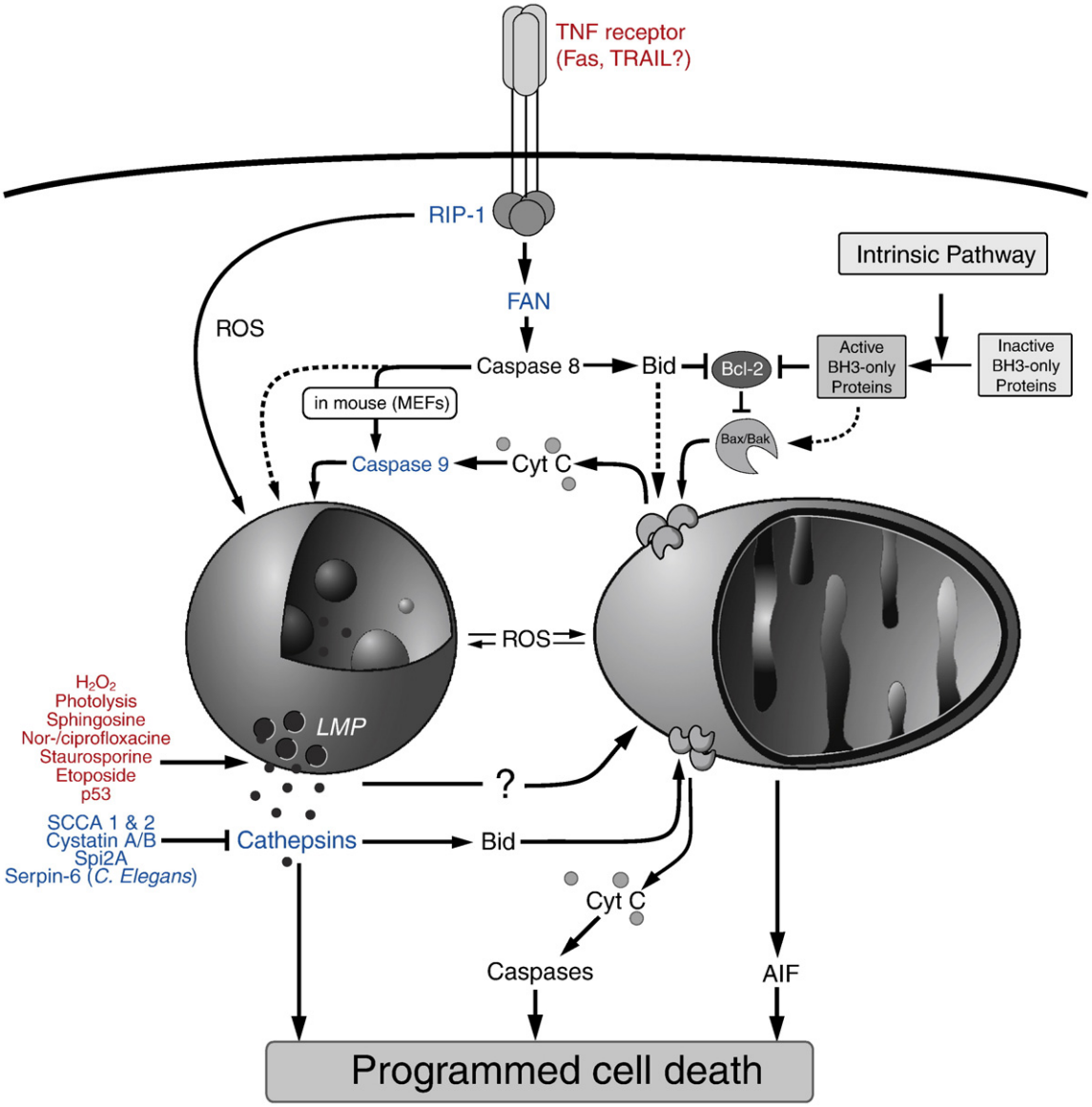

Fig. 2. Schematic presentation of lysosomal involvement in cell death signaling. Numerous cytotoxic treatments either directly or indirectly induce permeabilization of the lysosomal membranes (LMP), resulting in the release of lysosomal content into the cytosol. Here, e.g. lysosomal proteases, the cathepsins, can mediate cell death either in a caspase-independent manner or by inducing release of apoptogenic factors from the mitochondria followed by caspase activation (please refer to the text for details). Stimuli that induce LMP are printed in red. Proteins that modulate the lysosomal death pathways are printed in blue.

to LMP through the factor associated with neutral SMase (FAN)[64]. Studies based on FAN deficient immortalized MEFs as well as human fibroblasts expressing a dominant negative form of FAN have shown that FAN does not only mediate TNF-induced ceramide production, but also contributes to the caspase-8 processing and cell death. Since the TNF-induced LMP in murine hepatocytes depends on caspase-8, its reduced processing may explain the reduced LMP in TNF-treated hepatocytes expressing dominant negative FAN [8,59]. The role of ceramide and its metabolites can, however, not be ruled out. Their role in TNF-induced death signaling is supported by the reduced TNF and Fas-induced hepatotoxicity in mice deficient for aSMase, which is activated downstream of caspase-8[65,66]. Especially sphingosine that is generated from ceramide in a reaction catalyzed by the lysosomal enzyme acid ceramidase is a tempting candidate, as it, contrary to ceramide, can act as a detergent, directly destabilizing the lysosomal membrane [67,68]. In addition to increasing the generation of the sphingosine precursor, ceramide, by activating SMases, TNF regulates sphingosine levels also by cathepsin B-mediated down-regulation of sphingosine kinase-1, an enzyme that converts the pro-apoptotic sphingosine to an anti-apoptotic sphingosine-1-phosphate [69]. This activity of cathepsin B could result in the accumulation of sphingosine in the lysosomes and may thus, at least partially, explain the requirement of cathepsin B for an efficient LMP in TNF-treated hepatocytes [68].

TNF can also trigger LMP and cell death in the presence of caspase inhibitors. This pathway is independent of caspase-8, but requires the death domain-containing receptor interacting protein-1 (RIP-1) and involves the generation of reactive oxygen species [19,38,70,71] (T. Farkas and M. Jäättelä, unpublished data). Oxidative stress can, together with intralysosomal iron, generate oxygen radicals through a Fenton-type chemistry and thereby may cause oxidation of lysosomal membrane lipids, resulting in the destabilization of the membrane and the release of the lysosomal content [72,73]. The molecular links between RIP-1, oxidative stress and LMP are, however, still missing.

The induction of cell death by several classic apoptosis inducers (e.g. p53, etoposide and staurosporine) also involves LMP followed by cathepsin-dependent mitochondrial membrane permeabilization

[40,45,51,74]. However, the signaling pathways from these stimuli to LMP remain to be revealed.

2.3. Crosstalk between lysosomes and mitochondria

The cytotoxic effects of LMP often rely, at least partially, on the activation of the mitochondrial death pathway (Fig. 2). An elegant microinjection study has demonstrated that when localized to the cytosol, a single lysosomal hydrolase, cathepsin D, is sufficient to trigger the mitochondrial outer membrane permeabilization and apoptosis in human fibroblasts at cellular doses corresponding to half of the total cellular cathepsin D activity [75]. Cathepsin D is, however, not sufficient to trigger cell death in all models involving LMP. Other well-documented mediators of LMP-triggered cell death include cysteine cathepsins B and L as well as reactive oxygen species [76]. It should, however, be emphasized that possible roles for other lysosomal hydrolases, lysosome-derived second messengers and LMP-induced acidification of the cytosol are still open, not least so because of lack of research focusing on these questions. One of the links between cathepsins and mitochondrial membrane permeabilization may be Bid, a pro-apoptotic BH3-only protein of the Bcl-2 family that can be processed and activated by several cysteine cathepsins at cytosolic pH [36]. Along these lines, the aspartic Cathepsin D has been suggested to cleave and activate Bid in the acidic environment of the endolysosomal compartment following TNF receptor-1 (TNF-R1) internalization [77,78]. According to this model, the endocytosis of the ligand-activated TNF-R1 results in acid sphingomyelinase-mediated generation of ceramide, which then binds to the inactive cathepsin D and activates it via autocatalytic processing [79]. Cathepsin D may also activate Bax in a Bid-independent manner as demonstrated in staurosporine-treated T cells [51]. Furthermore, in fibroblasts treated with ciprofloxacine, LMP triggers mitochondrial membrane permeabilization through a Bid-independent activation of Bax and Bak [39]. In this model system the Bax activation is independent of cathepsin D, but relies instead on reactive oxygen species. It should be noted that ciprofloxacine-induced mitochondrial membrane permeabilization is not fully inhibited in cells lacking both Bax and Bak. The alternative mechanisms connecting LMP to the mitochondrial membrane permeabilization may include the direct effects of reactive oxygen species and/or lipid mediators such as arachidonic acid that can be generated in a cathepsin B-dependent manner [76].

2.4. Mitochondrion-independent lysosomal death pathways

Importantly, the lethal effects of LMP and cytosolic cathepsins are not limited to the activation of the intrinsic apoptosis pathway. In small cell lung cancer cells treated with microtubule stabilizing drugs (paclitaxel, epothilone B and discodermolide), LMP occurs early in the death process and cysteine cathepsins mediate micronucleation and cell death in a caspase-independent manner [46]. In TNF-treated human carcinoma cell lines LMP occurs downstream of mitochondrial outer membrane permeabilization [23,26,61]. However, the inhibition of cysteine cathepsin activity or expression confers significant protection against TNF-induced cell death without significantly inhibiting the effector caspase activation. Furthermore, cathepsin B is responsible for apoptosis-like changes, such as chromatin condensation, phosphatidylserine exposure and plasma membrane blebbing, in the absence of caspase activity in TNF-treated murine WEHI-S fibrosarcoma cells [23]. Furthermore, the depletion of heat shock protein 70 (Hsp70) in various human cancer cells [40] as well as supraoptimal activation of T cells [80] triggers LMP and cathepsin-mediated apoptosis-like cell death without the activation of the intrinsic apoptosis pathway. In line with these data, cathepsin B can induce nuclear apoptosis in isolated nuclei [81]. Thus, cathepsins appear to carry both the ability to act as initiator- as well as effector proteases of programmed cell death depending on the stimulus and the cellular context. Especially their ability to mediate cell death in cancer cells, where the mitochondrial death pathway is blocked for example due to overexpression of Bcl-2, raises hopes that treatments inducing LMP may prove effective in treatment of cancers that are resistant to inducers of classic apoptosis [22,40]. This idea is further supported by data showing that immortalization and transformation can sensitize cells to the lysosomal cell death [8,23,53,82]. One should be aware though, that the activity of cysteine proteases is not always positively correlated to cell death. It has, for instance, been shown that inhibitors of Cathepsin B such as CA-074 and E-64 lead to human neuroblastoma cell death dependent on caspases [83]. As such, the present understanding of the roles of cathepsins in programmed cell death appears to be context- and/or cell type-dependent, which could be attributed to functional redundancy of the cathepsins. A complimentary explanation for some of the discrepancies observed in the literature could be the site of action of the cathepsins as these proteases can act both intracellularly as well as extracellularly—this distinction becomes particularly important in cancer where cysteine cathepsins serve distinct pro-oncogenic extracellular roles, but in which one might also want to exploit their cytotoxic intracellular capabilities [9,10].

3. Transformation-induced changes in the lysosomal compartment

Transformation of cells leads to a series of changes in the lysosomal compartment which ultimately lead to increases in lysosomal volume and total protease activity as well as enhanced secretion of lysosomal proteases [9,84,85]. Studies on the lysosomal proteases, the cathepsins, have revealed that transformation induces marked changes in the trafficking and subcellular localization of the cathepsins B, D and L [86–90]. These changes in the lysosomal compartment become pro-oncogenic when the enhanced secretion of cysteine cathepsins initiate proteolytic pathways that increase neoplastic progression [9]. Indeed, particularly cathepsin B is recognized as an important contributor to tumor angiogenesis and the activity of this protease appears to be positively correlated with the metastatic potential of human pancreatic cancer [85]. However, also cathepsin S has been implicated in angiogenesis as a recent study on cathepsin S knockout mice demonstrated that this cathepsin can act as an enhancer of angiogenesis associated with wound healing in the skin [91].

Perhaps the most convincing evidence for the involvement of cathepsins in oncogenesis, however, come from mouse models of multistage carcinogenesis [92]. In a model of pancreatic islet tumorigenesis established by D. Hanahan [93], J.A. Joyce and co-workers profiled cysteine cathepsin expression and activity and found an elevation of cysteine cathepsin activity during tumor development, which was important for tumor angiogenesis, cell proliferation, tumor growth, and tumor invasion [92]. Further work from the group of J.A. Joyce has elaborated on these findings and defined distinct roles for a subset of the cysteine cathepsins (B, L and S) in the same model of multistage tumorigenesis [94]. Based on null-mutations of the cathepsins B, L, S and C it was shown that deficiency of cathepsins B or S impaired tumor formation and angiogenesis, whereas cathepsin B or L knockouts impeded cell proliferation and tumor growth. The absence of either cathepsin B, L or S impaired tumor invasion. Interestingly, the inhibition of cysteine cathepsin activity in this model using a membrane-permeable, pan-cathepsin-inhibitor, JPM-Oet, impaired angiogenic switching in progenitor lesions, as well as tumor growth, vascularity, and invasiveness and was later shown also to enhance chemotherapy regimens [92,95]. This is particularly interesting as lysosomal proteases, most notably cathepsin B, has also been implicated in tumor cell death programs (an overview is provided in Table 1) and is considered a possible chink in the armor which protects tumor cells against death.

In this regard, the primarily investigated way by which transformation-induced changes in the lysosomal compartment may serve anti-tumorigenic roles is in the context of induction of cell death. Even though cancer cells may have several blocks in their normal apoptotic cascades, tumor cell death may still be initiated via activation of the lysosomal compartment. Particularly during early stages of tumorigenesis, cancer cells are sensitized to numerous death stimuli and often undergo spontaneous cell death, possibly due to activation of oncogenes such as Myc and Ras, which can be sufficient to trigger cell death on their own or can sensitize the emerging cancer cells to various cytotoxic drugs or death-receptor activation [96]. In line with this, it has recently been shown that spontaneous immortalization sensitizes murine embryonic fibroblasts (MEFs) to a cysteine cathepsin-mediated lysosomal death pathway [53]. In this study, the susceptibility of wild-type MEFs to TNF mediated cytotoxicity increased more than 1000-fold upon immortalization, whereas immortalized MEFs deficient for cathepsin B retained the resistant phenotype of primary cells. In this model, the lack of another cysteine cathepsin, cathepsin L, also provided resistance to the sensitizing effect of immortalization to TNF, whereas lack of cathepsin D and caspase-3 had no effect on the TNF-sensitivity of the immortalized MEFs. Importantly, further oncogene-driven transformation of the immortalized MEFs was associated with a several-fold increase in cathepsin expression and additional sensitization to TNF and chemotherapeutic agents as well as decreased levels of LAMP-1 and –2 [53,128]. Importantly, in human cancer cell models, *K-ras* and *erbB2* elicit a similar activation of cysteine cathepsins and cathepsin-dependent downregulation of LAMPs. The sensitization to various lysosome-destabilizing drugs witnessed in these models most likely occur as a consequence of the cathepsin-mediated loss of the LAMPs as RNAi of either LAMP-1 or -2 is sufficient to sensitize the cells to lysosomal destabilization [53,128].

4. Cancer cell defenses against anti-tumorigenic lysosomal changes

Given the potential fatal outcome of lysosomal membrane permeabilization, it is not surprising that cells have developed numerous strategies to counteract it—either by inhibiting the lysosome membrane permeabilization itself or by protecting cells against the acid hydrolases leaking to the cytosol as a consequence of lysosomal rupture. This also holds true for cancer cells whose lysosomal compartment is particularly prone to destabilizing events [53].

4.1. Intracellular protease inhibitors

In the event of release of lysosomal proteases to the cytosol, cytosolic protease inhibitors present a bulwark against its deleterious consequences. When it comes to cancer, the best characterized intracellular lysosomal protease inhibitors are probably the squamous cell carcinoma antigens (SCCA) 1 and 2. These are tumor-associated proteins of squamous cell carcinomas of various organs and have been classified as serine protease inhibitors (serpin B3 and B4) whose levels are widely used for the diagnosis and management of diverse squamous cell carcinomas [97]. SCCA 1 and 2 are capable of inhibiting a variety of proteases including many of lysosomal origin such as cathepsins G, S, L and K and their inhibitory functions have been shown to confer tumor cell death resistance towards different apoptotic stimuli such as TNF and the activity of IL-2 activated NK-cells [97,98].

Whereas no endogenous inhibitors of cathepsin D are known, cysteine cathepsins can also be effectively inhibited by several other cytosolic protease inhibitors, i.e. cystatin A and B and serine protease inhibitor 2A (Spi2A) which was recently found to possess potent inhibitor activity also against several cysteine cathepsins (B, H, K, L and V) and cathepsin G [99–101]. The importance of these inhibitors in preventing cell death in physiological and pathological conditions is demonstrated by cystatin B-deficient mice which display increased apoptosis of cerebellar granule cells [102]. Moreover, the expression of Spi2A is induced upon TNF-treatment via the NF-κB pathway, and effectively inhibits TNF-induced cytosolic cathepsin B activity and cell death in MEFs [101,103].

Interestingly, it has just been reported that in *C. elegans*, the cytosolic serine protease inhibitor (serpin)-6 can protect against both the induction as well as the lethal effects from lysosomal injury caused by hypo-osmotic stress as well as a variety of other lysosomal stresses, demonstrating that protection against LMP is an evolutionarily conserved mechanism [33].

4.2. PI3K-signalling

Among its many other functions, phosphatidylinositol 3-kinase (PI3K) has been reported to protect lysosomes against destabilization. Inhibition of PI3K in human vascular endothelial cells induces the release of cathepsin B to the cytosol arguing for a rather direct role of PI3K in preserving lysosomal membrane integrity [44]. Furthermore, PI3K inhibitors sensitize the cells to the TNF- and interleukin-1-induced lysosomal death pathways [44]. Thus, PI3K, which is commonly activated in human cancer cells, may also contribute to lysosomal stability of tumor cells and thereby increase their cell death resistance.

4.3. Heat shock protein 70

Whereas the role of PI3K on the stability of tumor cell lysosomes at this point remains speculative, recent data advocate for a definite role for Heat shock protein (Hsp) 70 as a guardian of lysosomal integrity. This work has mainly been done in tumor cells, which also often demonstrate a localization of Hsp70 on the plasma membrane as well as in the endolysosomal compartment [40,104–108].

Apart from its anti-apoptotic abilities as a consequence of being a molecular chaperone, i.e. facilitating protein folding under otherwise denaturing conditions, Hsp70 is also able to enhance survival of cells in the face of a wide variety of stimuli including death-inducing cytokines and classical chemotherapeutic drugs [109–116].

A role for Hsp70 as an essential factor for cancer cell survival was first presented in a report by Wei et al., in a depletion-study of Hsp70 in cancer cells [117]. This role has since been substantiated in a series of experiments in which adenoviral antisense-mediated depletion of Hsp70 triggers a tumor cell-specific lysosomal death program [22,40]. *In vivo* studies utilizing orthotopic xenografts of glioblastoma and breast carcinomas as well as sub-cutaneous xenografts of colon-carcinoma in immunodeficient mice has further demonstrated the anti-cancer potential of Hsp70 depletion, as the tumors of mice receiving locoregional application of the adenoviral construct showed intracellular release of cathepsins, tumor cell death and recruitment of macrophages [118]. These studies clearly demonstrate the dependence of some tumors upon the presence of Hsp70, although parts of the observed cytotoxicity probably stem from the ability of the antisense construct to also target other Hsp70-family members such as Hsp70-2 [119–121]. Importantly, the cell death induced by the depletion of Hsp70 was not dependent on caspases, but was characterized by the release of cathepsins to the cytosol and inhibition of these provided significant cytoprotection [22,40], which suggests that parts of the potent cytoprotective effect of Hsp70 are due to stabilization of lysosomal membranes. Further evidence for this comes from studies which show that exogenous Hsp70 effectively inhibits lysosomal destabilization induced by various stresses [40,61,107] and that mice deficient for Hsp70 suffer from pancreatitis caused by the leakage of lysosomal proteases into the cytosol [122].

This cytoprotective mechanism of Hsp70, which many cancer cells seem to have adapted, i.e. the translocation of Hsp70 to the endolysosomal compartment [40] may have unfortunate consequences

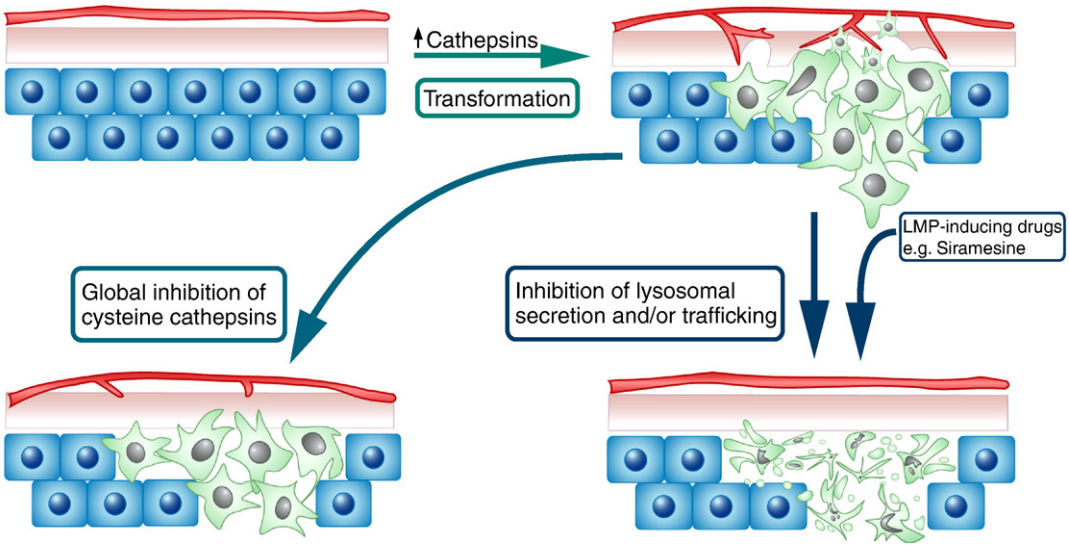

Fig. 3. Current and theoretical approaches to target and exploit the transformation-induced changes of the lysosomal compartment. During transformation several changes occur in the lysosomal compartment—amongst these increases in overall cathepsin activity. As lysosomal proteases both serve pro- as well as anti-oncogenic roles during tumor development via their extra- and intracellular functions, respectively, different approaches can be considered when it comes to target these for anti-cancer therapy. The global inhibition of cysteine cathepsins in a mouse model of pancreatic cancer has verified the feasibility of an "inhibit-all" approach [92] but in this way the pro-cell death functions of cathepsins are of course also inhibited. Alternatively, but yet only theoretically, the trafficking or secretion of lysosomes would present an obvious target. In this model, the inhibition of e.g. the secretory pathway would eliminate the extracellular pro-oncogenic properties of lysosomal proteases while increasing the intracellular amount available for cell death execution. As the lysosomal compartment furthermore becomes increasingly sensitive to disruptive stimuli upon transformation, this might render the cancer cells even more susceptible to lysosome-targeting anti-cancer agents such as Siramesine [125]. Additionally, the sheer bulk of "trapped" lysosomes might be enough to provide the amount of lysosomal protease release necessary for efficient cancer cell death to occur.

for the cancer cells however. Studies have shown that more than 50% of tumors show localization of Hsp70 on the plasma membrane surface [106] —an area which is directly connected with the endolysosomal compartment via endocytic and secretory events. Whereas the endolysosomal localized Hsp70 offers cytoprotection, the surface-exposed Hsp70 can act as a recognition structure for natural killer (NK) cells, stimulating their proliferation and cytolytic activity [105,123]. As the endolysosomal membranes and plasma membranes are constantly interchanged, the presence of Hsp70 on the surface of cancer cells could be an "unfortunate" consequence of two events that promote tumor progression; the secretion of cathepsins, which promotes invasion and angiogenesis, and the localization of Hsp70 on the lysosomal membranes, which prevents accidental release of cysteine cathepsins to the cytosol and ensuing cell death [92,124]. As such, these changes might all be parts of an ancient stress response triggered by the process of oncogenesis for better and for worse, although this remains speculative.

Whatever the primary cause of Hsp70 localization to the lysosomal compartment may be, the molecular mechanism for the cytoprotection this localization confers has so far remained elusive, but could provide several interesting targets for future cancer therapy.

5. Perspectives

The study of lysosomal changes in tumour progression and treatment is still very young, but the recent great advances in this field promise rapid progress in the near future. In this regard it is interesting that drug delivery should be very practicable for drugs targeting the lysosomes, as this compartment can be reached through the endocytic machinery. Other approaches targeting the lysosomes could include drugs targeting the already altered lysosomal trafficking pathways occurring in cancer cells, as this might give rise to populations of lysosomes even more prone to membrane rupture (Fig. 3).

Drug screens to identify molecules that induce lysosomal rupture may also prove effective in finding new compounds that activate the lysosomal death pathway. Interestingly, in a recent small molecule library screen, more than 50% of the compounds that induce significant cell death in p53-null cells trigger LMP and cathepsin-mediated killing of tumor cells [52]. Importantly, promising anti-cancer compounds targeting lysosomal integrity are also appearing such as the sigma-2 receptor ligand Siramesine [47,125].

At this point, the rapid advances in our understanding of cancer-associated changes in the lysosomes prompt additional investments in how to best target the pro- and anti-oncogenic potential of this complex compartment. In this regard, future elucidation of the molecular mechanisms controlling lysosomal membrane stability will hopefully present novel targets for cancer therapy.

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