Lysosomal cell death at a glance

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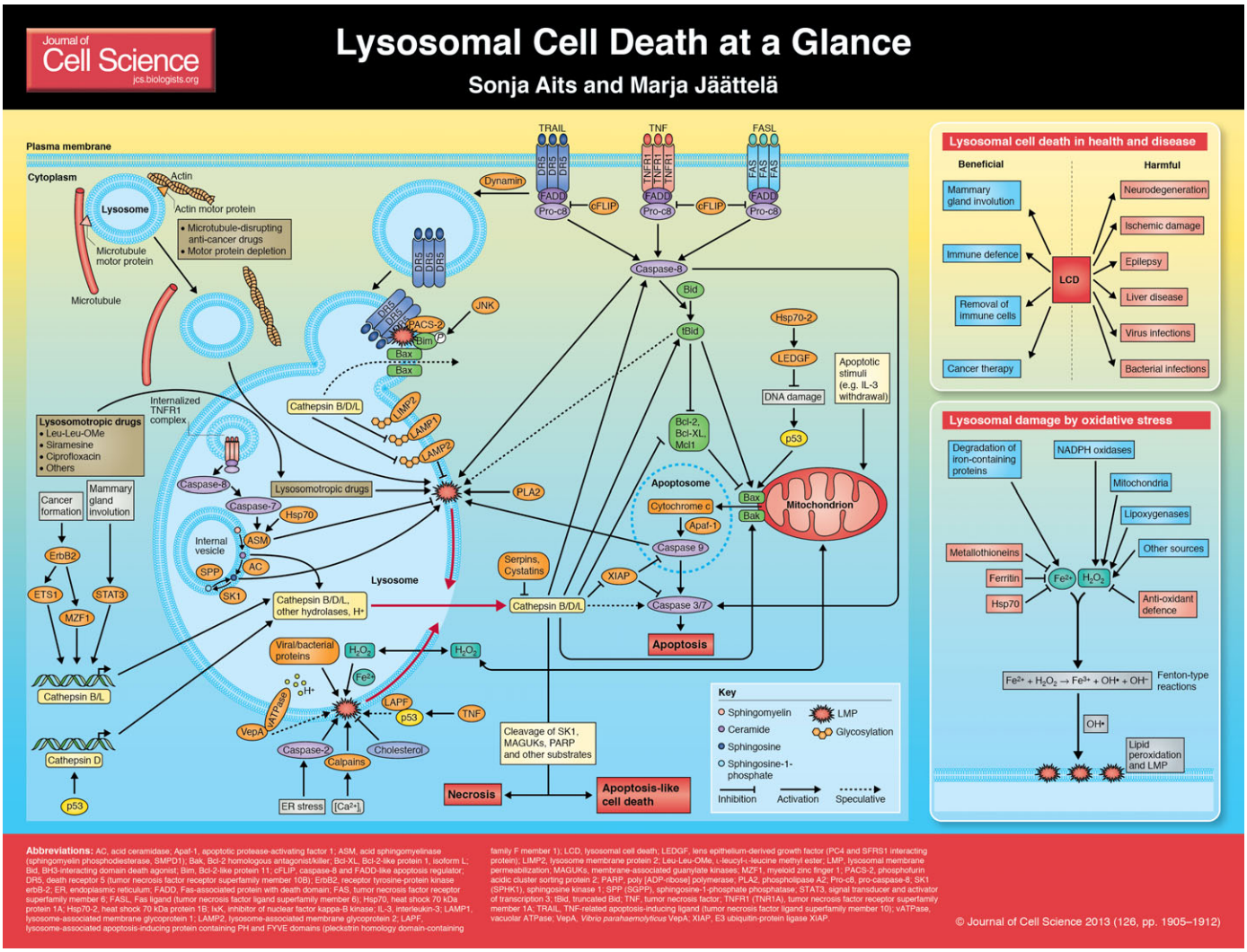
Summary

Lysosomes serve as the cellular recycling centre and are filled with numerous hydrolases that can degrade most cellular macromolecules. Lysosomal membrane permeabilization and the consequent leakage of the lysosomal content into the cytosol leads to so-called "lysosomal cell death". This form of cell death is mainly carried out by the lysosomal cathepsin proteases and can have necrotic, apoptotic or apoptosis-like features depending on the extent of the leakage and the cellular context. This article summarizes our current knowledge on lysosomal cell death with an emphasis on the upstream mechanisms that lead to lysosomal membrane permeabilization.

Introduction

The concept of lysosomal cell death (LCD) was first presented by Christian de Duve, who was awarded the Nobel Prize in 1974 for his discovery and characterization of lysosomes as cellular 'recycling bins'. Owing to the potent hydrolytic capacity of lysosomal enzymes, he also defined lysosomes as 'suicide bags' that can cause cell and tissue autolysis upon rupture (de Duve, 1983). Even though lysosomal rupture was recognized back in the 1970s as a powerful way to kill cells (Firestone et al., 1979), the interest in LCD faded during the following decades. This was largely due to the lack of methods to differentiate lysosomal rupture that causes cell death from post-death alterations in autolytic cells. Furthermore, lysosomal involvement in cell death was commonly overlooked because lysosomal membrane permeabilization (LMP) does not necessarily change the ultrastructure of lysosomes (Brunk and Ericsson, 1972) and because the ability of methyl-ketone-based protease inhibitors (e.g. zVAD-fmk) to inhibit cell death was generally considered as proof for caspase-mediated apoptotic cell death, even though such compounds also inhibit lysosomal cysteine cathepsins (Schotte et al., 1999). Thus, the



(See poster insert)

Table 1. Examples of genetically confirmed cellular models for lysosomal cell death

Stimulus	Cell type	Protective modification	LMP	References
Activators of death-receptor				
Anti-Fas	HeLa	*CTSD* antisense	n.d.	(Deiss et al., 1996)
TNF	WEHI-S	*Ctsb* antisense *CSTA* cDNA	Yes	(Foghsgaard et al., 2001)
	ME180as	*CTSB* antisense	Yes	(Foghsgaard et al., 2001)
	MEF	$Ctsd^{-/-}$	n.d.	(Heinrich et al., 2004)
TNF plus actinomycin D	Murine hepatocytes	$Ctsb^{-/-}$	Yes	(Guicciardi et al., 2005; Guicciardi et al., 2000)
TNF plus cycloheximide	MEF	$Ctsb^{-/-}$ $Ctsl^{-/-}$	Yes	(Fehrenbacher et al., 2004)
	$Rela^{-/-}$ MEF	*Spi2A* cDNA	Yes	(Liu et al., 2003)
TRAIL	Huh-7	*CTSB* shRNA	Yes	(Guicciardi et al., 2007)
	KMCH-shMcl	*CTSB* shRNA	Yes	(Werneburg et al., 2007)
DNA-damaging agents				
Adriamycin	MEF	$Ctsd^{-/-}$	n.d.	(Wu et al., 1998)
Etoposide	MEF	$Ctsd^{-/-}$	n.d.	(Wu et al., 1998)
	U937	*CTSD* siRNA	Yes	(Emert-Sedlak et al., 2005)
	Murine monocytes	$Ctsb^{-/-}$	Yes	(Oberle et al., 2010)
Viruses and bacteria				
Nef, HIV-1	Human $CD4^{+}$ T cells	*CTSD* siRNA	Yes	(Laforge et al., 2007)
Parvovirus H-1	NCH82	*CTSB* siRNA *CTSL* siRNA	Yes	(Di Piazza et al., 2007)
VepA, *Vibrio parahaemolyticus*	HeLa	*ATP6V0C* siRNA	Yes	(Matsuda et al., 2012)
	Saccharomyces cerevisiae	$VMA3^{-/-}$	Yes	(Matsuda et al., 2012)
Other stimuli				
Interferon-γ	HeLa	*CTSD* antisense	n.d.	(Deiss et al., 1996)
Staurosporine	Human T cells	*CTSD* siRNA	Yes	(Bidère et al., 2003)
Spontaneous death	Murine neutrophils	$Ctsd^{-/-}$	Yes	(Conus et al., 2008)
Leu-Leu-OMe	Murine breast cancer cells	$Ctsb^{-/-}$	n.d.	(Vasiljeva et al., 2008)
Granulysin	HeLa	*CTSB* shRNA	Yes	(Zhang et al., 2009)
Withdrawal of interleukin-3	Murine monocytes	$Ctsb^{-/-}$ $Ctsl^{-/-}$	Yes	(Oberle et al., 2010)

Cell types: HeLa, human cervix carcinoma; Huh-7, human hepatocellular carcinoma; KMCH-shMcl, Mcl-1-depleted human KMCH cholangiocarcinoma; MEF, murine embryonic fibroblast; ME-180as, Hsp70-depleted ME-180 human cervix carcinoma; NCH82, human glioma; U937, human histiocytic lymphoma; WEHI-S, TNF-sensitive subclone of WEHI-164 murine fibrosarcoma.

Genes: *ATP6V0C*, human ATPase, H+ transporting, lysosomal 16kDa, V0 subunit c; *CSTA*, human cystatin A; *Ctsb*/*CTSB*, murine/human cathepsin B; *Ctsd*/*CTSD*, murine/human cathepsin D; *Ctsl*/*CTSL*, murine/human cathepsin L; Rela, v-rel reticuloendotheliosis viral oncogene homolog A (avian); *Spi2A*, murine serine protease inhibitor 2A (serine (or cysteine) peptidase inhibitor, clade A, member 3G, Serpina3g); *VMA3*, *S. cerevisiae* V-type ATPase V0 subunit c.

Abbreviations: LMP, lysosomal membrane permeabilization; n.d., not determined; Leu-Leu-OMe, L-leucyl-L-leucine methyl ester.

interest in LCD was revived only recently when more advanced assays to study LMP were developed and emerging genetic data corroborated the role of cathepsins as evolutionarily conserved executors of cell death (Tables 1 and 2). This article and the accompanying poster briefly summarize the molecular mechanisms of LCD.

Induction of LMP

Most, if not all, cell death pathways eventually lead to LMP (Vanden Berghe et al., 2010). To define LCD, it is thus important to differentiate between LMP that is required for cell death and LMP that is a consequence of it. Tables 1 and 2 list experimental systems in which the role of lysosomes in causing cell death has been confirmed. Additionally, numerous other stimuli, including most known inducers of apoptosis, can trigger LMP that either initiates or amplifies the cell death program (Groth-Pedersen and Jaattela, 2010; Johansson et al., 2010). Except for lysosomotropic detergents (detergents that accumulate in lysosomes) and pore-forming toxins, the mechanisms underlying LMP are largely ambiguous, possibly reflecting multiple means to permeabilize the lysosomal membrane, as discussed below.

Lysosomotropic detergents

Lysosomotropic detergents damage the lysosomal membrane owing to their detergent-like properties (de Duve et al., 1974; Firestone et al., 1979). They are weak bases that diffuse across membranes and become trapped in the acidic lysosomes after protonation (de Duve et al., 1974). Examples of lysosomotropic detergents include amines with hydrophobic side-chains (e.g. imidazole and morpholine) (Firestone et al., 1979), ciprofloxacin (Boya et al., 2003), *O*-methyl-serine dodecylamide hydrochloride (Li et al., 2000), sphingosine (Kågedal et al., 2001) and siramesine (Ostenfeld et al., 2008), all of which are potent inducers of LMP. Although most lysosomotropic detergents are likely to be cytotoxic to all lysosome-bearing cells (Firestone et al., 1979), the transformation-associated sensitization to some of them (e.g. siramesine) opens possibilities for their use in cancer therapy (Ostenfeld et al., 2005). In addition, L-leucyl-L-leucine methyl ester (Leu-Leu-OMe) is under development for the treatment of graft-versus-host disease owing to its pronounced effect on cytotoxic lymphocytes. The increased sensitivity of these cells depends on their high level of cathepsin C, which is required to convert Leu-Leu-OMe into the detergent $(Leu\text{-}Leu)_n$-OMe ($n>3$) after its delivery to the lysosomes by receptor-mediated endocytosis (Uchimoto et al., 1999).

Viral proteins

Virus infection requires the delivery of viral genes into the cell, which mostly occurs by penetrating the endolysosomal membranes with viral entry proteins that

Table 2. Examples of *in vivo* models of lysosomal cell death

Stimulus	Species	Tissue	Model for	Rescue	References
IRI	*Macaca fuscata*	Hippocampus	Stroke	CA-074	(Yamashima et al., 1998)
Caerulein	*Mus musculus*	Pancreas	Acute pancreatitis	*Ctsb*$^{-/-}$	(Halangk et al., 2000)
Cstb$^{-/-}$	*Mus musculus*	Cerebellum	Unverricht-Lundborg epilepsy	*Ctsb*$^{-/-}$	(Houseweart et al., 2003)
IRI	*Mus musculus*	Liver	Liver transplantation	R3032	(Ben-Ari et al., 2005)
Bile duct ligation	*Mus musculus*	Liver	Cholestasis	*Ctsb*$^{-/-}$	(Canbay et al., 2003)
LPS	*Mus musculus*	Neutrophils	Sepsis	*Ctsd*$^{-/-}$	(Conus et al., 2008)
Weaning	*Mus musculus*	Breast	Mammary gland involution	CA-074Me, *Stat3*$^{-/-}$	(Kreuzaler et al., 2011)
TNF plus Ad5IκB	*Mus musculus*	Liver	Hepatitis	*Ctsb*$^{-/-}$	(Guicciardi et al., 2000; Guicciardi et al., 2001)
norpA$^{-/-}$	*Drosophila melanogaster*	Eye	Retinal degeneration	*Cp1* mutant, ectopic *Cys*, ectopic *Spn4*	(Kinser and Dolph, 2012)
Hypoxia, Ca^{2+}	*Caenorhabditis elegans*	Neurons	Stroke, neurodegeneration	*asp-3/4*$^{-/-}$, pepstatin A, lysosomal alkalization	(Artal-Sanz et al., 2006; Syntichaki et al., 2002)
srp-6 RNAi plus hypo-osmotic stress	*Caenorhabditis elegans*	Intestines	Gastrointestinal stress	*asp-1/3*$^{-/-}$, ectopic *srp-6*, E64d	(Luke et al., 2007)
Chloroquine	*Plasmodium falciparum*	Parasite	Malaria	E64d, zFA-fmk	(Ch'Ng et al., 2010; Ch'Ng et al., 2011)

Abbreviations: Ad5IκB, adenovirus expressing IκB (Nfkbia) superrepressor S32A/S36A mutant; CA-074Me and E64d, cysteine cathepsin inhibitors; IRI, ischemia-reperfusion injury; LPS, lipopolysaccharide; pepstatin A, cathepsin D inhibitor; R3032, cathepsin B inhibitor; zFA-fmk, cysteine cathepsin inhibitor.
Genes: *asp-1/3/4*, *C. elegans* cathepsin D/E-like; *Cp1*, *D. melanogaster* cathepsin L-like; *Cstb*, murine cystatin B; *Ctsb*, murine cathepsin B; *Ctsd*, murine cathepsin D; *Cys*, D. melanogaster cystatin-like; *norpA*, *D. melanogaster* phospholipase C; *Spn4*, *D. melanogaster* serpin 4 (protease inhibitor); *srp-6*, *C. elegans* serpin 6; Stat3, murine signal transducer and activator of transcription 3.

become active in the acidic environment (Lozach et al., 2011; Vázquez-Calvo et al., 2012). The penetration of non-enveloped viruses is typically achieved by endolysosomal membrane rupture (e.g. adenovirus and rhinovirus HRV14) or pore formation (e.g. rhinovirus HRV2 and poliovirus) (Prchla et al., 1995), which also releases lysosomal content into the cytosol. Adenovirus membrane lytic protein VI ruptures the membrane by causing membrane curvature stress (Maier et al., 2010; Wiethoff et al., 2005), but membrane rupture can also be caused by vesicular swelling beyond the retaining capacity of the membrane. Alternatively, viral capsid proteins of HRV2 and poliovirus insert directly into the endolysosomal membrane and form size-selective pores (Fuchs and Blaas, 2010; Tosteson and Chow, 1997). By contrast, parvovirus H-1 induces lethal LMP in glioma cells that is not directly related to the viral entry process but instead results from a dramatic downregulation of cytosolic cysteine cathepsin inhibitors, which sensitizes the cells to otherwise non-lethal cathepsin release (Di Piazza et al., 2007).

The entry of enveloped viruses has not been associated with LMP, possibly owing to the ability of the viral envelope to seal the endolysosomal membrane. Nevertheless, proteins of these viruses that are not involved in the entry process can induce lethal LMP. HIV-1 Nef causes LMP when expressed in high amounts in the cytosol, which might contribute to the massive destruction of CD4-positive T cells upon HIV-1 infection (Laforge et al., 2007). In addition, viral cationic peptides (e.g. HIV-1 Tat peptide), which, upon protonation in the acidic environment, acquire detergent-like properties, might damage lysosomes (Meade and Dowdy, 2007; Ziegler et al., 2005).

Bacterial, fungal and snake toxins

In a manner similar to viral entry proteins, many bacterial toxins form pores after undergoing conformational changes at low pH (Kagan et al., 1981; Sandvig and van Deurs, 2005). Accordingly, many of them strongly induce LCD, including *Bacillus anthracis* toxin (Newman et al., 2009), *Streptomyces hygroscopicus* nigericin (Hentze et al., 2003), *Pseudomonas aeruginosa* pyocyanin (Prince et al., 2008) and *Aggregatibacter actinomycetemcomitans* leukotoxin (DiFranco et al., 2012). Similarly, the cytotoxicity of enniatin mycotoxins (Ivanova et al., 2012), and venom toxins from cobra (Feofanov et al., 2005) and South American rattlesnake (Hayashi et al., 2008), have been connected with LMP. Additionally, *Vibrio parahaemolyticus* VepA was recently identified as a new type of LMP-inducing protein (Matsuda et al., 2012). After inoculation, VepA binds to the cytoplasmic tail of the channel-forming subunit c of vacuolar H^+-ATPase and triggers leakage of lysosomal hydrolases into the cytosol in a manner that depends on the subunit c. It will be of great interest to investigate whether VepA causes the widening of the ATPase channel and whether other LMP-inducing stimuli utilize a similar mechanism.

Reactive oxygen species

Reactive oxygen species (ROS) contribute to LMP that is induced by a wide range of oxidative stimuli (e.g. drugs, heavy metals and ionizing radiation) and conditions (e.g. ischemia–reperfusion injury, inflammation and neurodegenerative disorders) (Kurz et al., 2008a). Upon oxidative stress, excess H_2O_2 diffuses into lysosomes, where it reacts with redox-active iron, resulting in the production of hydroxyl radicals in Fenton-type reactions (see Poster) (Kurz et al., 2008b). Hydroxyl radicals are highly reactive and can destabilize the lysosomal membrane by causing lipid peroxidation and damaging lysosomal membrane proteins. Additionally, ROS might contribute to LMP by activating lysosomal Ca^{2+} channels (Sumoza-Toledo and Penner, 2011) or altering the activity of lysosomal enzymes such as phospholipase A2 (PLA2). In concordance with the lysosome-destabilizing effect of ROS, various antioxidants and redox regulators as well as iron-binding proteins confer protection against oxidative-stress-induced LMP (Kurz et al., 2008a; Kurz et al., 2008b).

Proteases

Cathepsins are mainly considered to be downstream mediators of LCD, but they can apparently also initiate LMP. Supporting this hypothesis, lack of cathepsin B prevents LMP in hepatocytes treated with tumor necrosis factor (TNF) or sphingosine (Werneburg et al., 2002). Furthermore, sensitization to LMP upon oncogene-driven transformation and several models of LCD (e.g. mammary gland involution and death induced by cytoskeletal disruption) are associated with increased cysteine cathepsin activity (Fehrenbacher et al., 2008; Fehrenbacher et al., 2004; Kreuzaler et al., 2011; Groth-Pedersen et al., 2007; Groth-Pedersen et al., 2012). The LMP-promoting effect of cysteine cathepsins might be due to the intralysosomal degradation of highly glycosylated lysosome-associated membrane proteins, which form a protective glycocalyx shield on the inner lysosomal membrane (Eskelinen et al., 2003; Fehrenbacher et al., 2008). Alternatively, minor leakage of cathepsins could activate LMP by cleaving sphingosine kinase 1 or other cytosolic substrates that maintain lysosomal stability (Mora et al., 2010; Taha et al., 2005).

Other proteases can also cause LMP. Cytosolic calpain proteases contribute to LMP upon ischemic and hypochlorous-acid-induced injury of neurons (Windelborn and Lipton, 2008; Yamashima et al., 1998; Yap et al., 2006). After deprivation of oxygen and glucose, μ-calpain localizes to lysosomes in hippocampal slices, suggesting a direct effect on the lysosomal membrane (Yamashima et al., 1996). Interestingly, heat shock protein 70 (Hsp70), which stabilizes lysosomes, has been proposed to be a target of calpain in this context (Yamashima, 2012).

Finally, the activation of apoptotic caspases is frequently associated with secondary LMP that might speed up or amplify the death process. Often, such secondary LMP is initiated by caspase-9, which can be activated in the apoptosome or, in murine cells, by caspase-8-dependent cleavage (Gyrd-Hansen et al., 2006; Oberle et al., 2010). Furthermore, caspase-2 has been reported to cause LMP and subsequent activation of other caspases in tunicamycin-treated leukemia cells (Huang et al., 2009). The caspase targets that are responsible for LMP remain mostly speculative (Oberle et al., 2010). After TNF receptor internalization, cathepsin D release can result from a caspase-8 and -7-dependent cascade that activates acid sphingomyelinase (ASM; see below) (Edelmann et al., 2011; Tchikov et al., 2011). Additionally, TNF-induced LMP in hepatocytes has been reported to be partially inhibited in the absence of the caspase-8 target Bid (Guicciardi et al., 2005; Werneburg et al., 2004), a BH3-only protein, whose truncated form (tBid) is essential for TNF-induced activation of pore-forming Bcl-2 proteins (Bax and Bak) and subsequent mitochondrial outer membrane permeabilization (MOMP) (Happo et al., 2012). It is unclear, however, whether tBid initiates LMP directly or whether it promotes LMP by means of MOMP (Happo et al., 2012).

Lipids and their metabolites

The sphingolipid metabolite sphingosine may act as an endogenous lysosomotropic detergent following treatments that induce its accumulation – for example, through the activation of lysosomal ASM and acid ceramidase in TNF-treated rat hepatocytes (Ullio et al., 2012). ASM might also enhance the LCD pathway through ceramide-mediated activation and release of cathepsin D (Heinrich et al., 2004; Heinrich et al., 1999). By contrast, ASM activity protects cells against photooxidation-induced LMP, and this might explain the potent lysosome-stabilizing effect of Hsp70, which enhances ASM activity by promoting its binding to lysosomal membranes (Kirkegaard et al., 2010; Nylandsted et al., 2004). Notably, LMP is also triggered by inhibition of sphingosine kinase 1, which converts sphingosine to sphingosine-1-phosphate (S1P) (Mora et al., 2010). In this case, however, loss of S1P, rather than accumulation of sphingosine, damages the lysosomes by hindering lysosomal recycling. Interestingly, sphingosine kinase 1 is a cathepsin B substrate (Taha et al., 2005), whose degradation might contribute to the amplification of LMP. Overall, lysosomal sphingomyelin catabolism controls lysosomal stability by multiple means, with the excess of either sphingomyelin or sphingosine having a destabilizing effect and S1P preserving normal lysosomal function.

LMP can also be caused by phospholipase A2 (PLA2), which has been shown to destabilize purified lysosomes (Zhao et al., 2003). Based on studies with semi-selective pharmacological PLA2 inhibitors, cytosolic PLA2 has been implicated in LCD induced by low concentrations of H_2O_2 (Zhao et al., 2001), neuronal ischemia (Windelborn and Lipton, 2008) and TNF (Wissing et al., 1997), whereas secretory PLA2 has been associated with LCD induced by heavy metals and environmental pollutants (Marchi et al., 2004). These effects might be mediated by arachidonic acid, a lipid metabolite generated by PLA2, which displays detergent-like properties and increases lysosomal permeability to K^+ and H^+, thereby enhancing lysosomal osmotic sensitivity (Zhang et al., 2006). Thus, PLA2 activity could contribute to LMP in several ways, but more research is required to clarify how different PLA2 enzymes promote LMP.

Loss of cholesterol might also increase lysosomal permeability to K^+ and H^+ and thereby destabilize the lysosomes (Johansson et al., 2010), but this effect is still poorly understood.

p53

Even though LMP can occur in the absence of cellular tumor antigen p53 (Erdal et al., 2005; Nylandsted et al., 2000; Ostenfeld et al., 2005), emerging evidence supports the notion that p53 can trigger LMP. For example, in myeloid leukemia cells, the activation of temperature-sensitive p53 is sufficient to cause LMP that precedes MOMP (Yuan et al., 2002). Furthermore, early LMP in TNF-treated fibrosarcoma cells (Li et al., 2007), embelin-treated colon cancer cells (Joy et al., 2010), as well as in cortical neurons exposed to Δ9-tetrahydrocannabinol or β-amyloid (Fogarty et al., 2010; Gowran and Campbell, 2008) depends on p53 and is associated with the localization of phospho-Ser15-p53 to the lysosomal membrane. The recruitment of phospho-Ser15-p53 to the lysosomes depends on LAPF (LMP-inducing lysosome-associated apoptosis-inducing protein containing PH and FYVE domains) (Li et al., 2007). It will be of great interest to reveal the mechanism of action of these proteins and to investigate whether p53 and/or LAPF link other cellular signals to LMP.

Proapoptotic Bcl-2 family members

Proapoptotic Bcl-2 family members are not essential for the induction of LMP, as demonstrated by the failure of Bcl-2 overexpression or Bax–Bak double-deficiency to prevent LMP after various stimuli (Boya et al., 2003; Gonzalez et al., 2012; Gyrd-Hansen et al., 2006;

Nylandsted et al., 2000; Ostenfeld et al., 2005; Rammer et al., 2010). They might, however, contribute to LMP in some model systems, as discussed above for the role of tBid in TNF-treated hepatocytes. Besides, it has been suggested that Bim recruits activated Bax to the lysosomes and thereby promotes LMP in hepatocytes treated with TRAIL (TNF-related apoptosis-inducing factor) (Werneburg et al., 2012; Werneburg et al., 2007). Even though Bax can form pores in isolated lysosomes *in vitro* (Kågedal et al., 2005), its lysosomal localization and direct involvement in LMP remains, however, controversial (Oberle et al., 2010; Repnik et al., 2012). Moreover, a recent report has revealed an unexpected role for Bim in lysosomal acidification (Ruppert et al., 2012), which might indirectly contribute to LMP.

Other regulators

The disruption of cytoskeleton and cellular trafficking by microtubule-targeting drugs (Bröker et al., 2004; Groth-Pedersen et al., 2007) or by depletion of cytoskeleton-associated motor proteins (Groth-Pedersen et al., 2012) also induces LMP. However, the underlying mechanisms are poorly understood. In addition, many other molecules regulate LMP, as reviewed elsewhere (Boya and Kroemer, 2008; Kirkegaard and Jäättelä, 2009; Kroemer and Jäättelä, 2005; Repnik et al., 2012).

Overall, a large number of stimuli and mediators have been implicated in LMP, but future work is likely to connect many of them to a lesser number of signalling pathways that converge on even fewer mechanisms actually causing LMP.

Consequences of LMP

It is unclear whether the entire lysosomal population is equally prone to LMP or whether a subpopulation of lysosomes is specifically targeted by LMP-inducing stimuli. It is, however, clear that the extent of LMP determines the morphological features of cell death. Extensive LMP results in uncontrolled necrosis with rapid plasma membrane permeabilization, whereas limited LMP can activate the intrinsic apoptosis pathway in apoptosis-competent cells (Kågedal et al., 2001) or caspase-independent death with apoptosis-like morphology in cells with defective apoptosis (Kirkegaard and Jäättelä, 2009).

In the case of extensive LMP, most lysosomal content leaks into the cytosol, and specific inhibitors of lysosomal hydrolases fail to attenuate cell death. By contrast, inhibition of cathepsins – especially cysteine cathepsins B and L and aspartyl cathepsin D – by genetic or pharmacological targeting or by overexpression of cytosolic cathepsin inhibitors (e.g. cystatin A or serine protease inhibitor 2A) can confer significant protection against cell death following limited LMP (Tables 1 and 2). The role of cathepsins as executors of LMP-induced apoptosis and apoptosis-like cell death is further supported by the ability of microinjected cathepsin B or D to trigger MOMP and apoptosis (Bivik et al., 2006; Roberg et al., 2002) as well as the capability of cathepsin B to induce apoptotic morphology in isolated nuclei (Vancompernolle et al., 1998).

LMP-induced apoptosis is usually activated through MOMP, which can be brought about by cathepsin-mediated activating cleavage of pro-apoptotic (Bid) or inhibiting cleavage of anti-apoptotic Bcl-2 proteins (Bcl-2, Bcl-X_L and Mcl-1) (Appelqvist et al., 2012; Cirman et al., 2004; Droga-Mazovec et al., 2008). Furthermore, cytosolic cathepsins can activate apoptotic caspases by cleaving either them or their inhibitor E3 ubiquitin-protein ligase XIAP (Conus et al., 2008; Droga-Mazovec et al., 2008; Vancompernolle et al., 1998; Zhou and Salvesen, 1997). The activated caspases can then enhance either MOMP-dependent or -independent apoptotic death.

Notably, LMP can also cause cell death with little or no caspase activation – for example, in response to hypochlorous acid (Yap et al., 2006), depletion of Hsp70 (Nylandsted et al., 2000), antibodies to CD3 (Michallet et al., 2004) or siramesine (Ostenfeld et al., 2005) – and, even when caspases are activated, their inhibition does not necessarily reduce cell death (Di Piazza et al., 2007; Nylandsted et al., 2004). Instead, cathepsins themselves can cleave many cellular proteins and take over the role of 'death-executing proteases' (Turk et al., 2012). So far, only a few cell death-promoting cathepsin substrates have been identified (Turk et al., 2012). As discussed above, sphingosine kinase 1 might be one of them. Additionally, cathepsins can cleave the caspase substrate PARP (Gobeil et al., 2001) and cell adhesion molecules such as membrane-associated guanylate kinases (MAGUKs), thereby inducing cellular detachment (Ivanova et al., 2011).

It should be emphasized that, even though cathepsins are important executors of LCD, their inhibition provides only partial protection from LCD. Thus, more studies are clearly needed to define the roles of other lysosomal hydrolases (e.g. lipases and phosphatases), lysosome-derived second messengers (e.g. Ca^{2+}, H^+ and ROS) and LMP-associated lysosomal dysfunction in LCD.

Perspectives

LCD has long been overlooked as a mode of regulated cell death. Nevertheless, its regulation and tight links to other cell death pathways are finally beginning to emerge. As discussed above and reviewed elsewhere (Boya and Kroemer, 2008; Česen et al., 2012; Kirkegaard and Jäättelä, 2009; Yamashima and Oikawa, 2009), LCD has important physiological functions, and it contributes to numerous degenerative and infectious diseases (see Poster). Nevertheless, it might provide an alternative strategy for the treatment of apoptosis- and multidrug-resistant cancers (Groth-Pedersen and Jaattela, 2010; Kallunki et al., 2012; Kreuzaler and Watson, 2012). However, a great amount of basic research is still needed to bring our knowledge of the complex regulation of lysosomal stability up to a level that allows the optimal design of LCD-targeting therapies.

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A high-resolution version of the poster is available for downloading in the online version of this article at jcs.biologists.org. Individual poster panels are available as JPEG files at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.091181/-/DC2

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