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Review

Cathepsins: Key modulators of cell death and inflammatory responses

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ABSTRACT

Apoptosis is a key mechanism in the build up and maintenance of both innate and adaptive immunity as well as in the regulation of cellular homeostasis in almost every organ and tissue. Central to the apoptotic process is a family of intracellular cysteine proteases with aspartate-specificity, called caspases. Nevertheless, there is growing evidence that other non-caspase proteases, in particular lysosomal cathepsins, can play an important role in the regulation of apoptosis. In this review, the players and the molecular mechanisms involved in the lysosomal apoptotic pathways will be discussed as well as the importance of these pathways in the immune system and the pathogenesis of diseases.

Contents

1. Introduction

The immune system is a remarkably adaptive defense mechanism that has evolved in vertebrates to protect them from invading infectious agents and to repair damaged tissue. It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently unlimited variety of foreign invaders. Although accumulation of these specialized cells and molecules at inflammatory sites helps to efficiently eliminate invading agents, it may also amplify the inflammatory response by damaging surrounding tissue [1]. Therefore, mechanisms are

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needed to tightly control the number of inflammatory cells, in particular under inflammatory conditions, during which stimulated cells are releasing toxic mediators.

Apoptosis, or programmed cell death, is an innate mechanism by which an organism eliminates such unwanted and potentially harmful cells from inflamed tissues without releasing hazardous intracellular contents [2,3]. In contrast to necrosis, apoptosis is the most physiological form of cell death [4,5]. Apoptotic cells die via two main pathways: the extrinsic and the intrinsic apoptotic pathways. The intrinsic pathway is triggered by a whole variety of intracellular stress signals such as growth factor withdrawal, aberrant calcium flux, viral infection, oxidative stress, UV radiation or cytotoxic agents, mediating mitochondrial outer membrane permeabilization (MOMP) which in turn induces the release of apoptogenic factors from the intermitochondrial space in order to activate downstream effectors. The extrinsic apoptotic pathway is induced by ligand-mediated stimulation of members belonging to the tumor necrosis factor (TNF)/nerve growth factor (NGF) superfamily of cell-surface 'death receptors' (e.g. Fas/CD95, TNF-receptor I or TNFRI). These receptors contain, within their cytoplasmic region, a functional death domain (DD) which is able, via adapter proteins, to trigger an autocatalytic activation cascade leading to apoptosis.

For both pathways, the central component is a proteolytic cascade involving proteases called caspases. Caspases are a family of evolutionarily conserved cysteinyl proteases mediating initiation and execution of apoptosis through aspartate-specific cleavage of a wide number of cellular substrates [6]. This very specific cleavage either activates their substrates, such as certain apoptosis-related endonucleases and protein kinases, or inactivates them, such as anti-apoptotic proteins, transcription factors, mRNA splicing proteins, translation initiators, or cytoskeletal components [7].

Besides caspases, another family of proteases, namely cathepsins, has recently been shown to be associated with cell death regulation [8–11]. Although cathepsins have often been considered as intracellular proteases capable of mediating caspase-independent cell death [8], there is also evidence that they act in concert with caspases in apoptotic cell death. In this review, we will discuss the role of cathepsins in caspase-dependent and -independent apoptotic pathways and hypothesize that lysosomes, in which cathepsins are located within healthy cells, could be a new therapeutic target in immune responses by regulating the life span of inflammatory cells like neutrophils and T or B cells. In addition, we will review a panel of molecules which are able to either permeabilize or stabilize the lysosomal membrane, such as certain chemotherapeutic drugs, reactive oxygen species (ROS), Bcl-2 family members and heat shock proteins.

2. Cathepsins: activity, function and the agents inducing or inhibiting their release from lysosomes

Since 1920, the term ''cathepsin'' stands for lysosomal proteolytic enzyme regardless of the enzyme class. Thus, this term includes serine proteases (cathepsins A and G), aspartic proteases (cathepsin D and E) as well as the eleven known human cysteine cathepsins (cathepsins B, C, F, H, K, L, O, S, V, X and W) (Table 1). Cathepsins are synthesized as inactive proenzymes, glycosylated post-translationally, and directed towards the lysosomal compartment by means of cellular mannose-6-phosphate receptors [12]. The processing of the inactive proenzyme into a catalytically active enzyme usually occurs within the lysosome [13]. Therefore, when released from the lysosome, cathepsins are fully active and do not require any further conformational change. Most of the cathepsins are endopeptidases, with the exception of the exopeptidases cathepsins C and X. The cathepsin activity is regulated by several mechanisms such as regulation of synthesis, zymogen processing, inhibition by endogenous inhibitors (e.g. stefins and cystatins for cysteine cathepsins) and pH stability [14,15]. An important question that arises due to a significantly lower pH found in lysosomes is whether cathepsins are able to retain their activity at physiological pH following release in the cytosol. Although cathepsins are still

Table 1 – Lysosomal cathepsins and their targets

Cathepsin	Cytosolic substrates	Involvement in immune function
Serine protease		
Cathepsin G	PARP-1	Cancer, induction of apoptosis in Jurkat T cells
Aspartic protease		
Cathepsin D	Bid, Bax, caspase-8	Selectors for the repertoire of surface peptide/MHC II complexes, induction of apoptosis in neutrophils, rheumatoid arthritis, cancer
Cysteine proteases		
Cathepsin B	Bid, Bak, BimEL, Bcl-2, Bcl-xL, Mcl-1, caspase-1/-2/-11, PARP-1, XIAP, E-cadherin	Induction of apoptosis in T and B cells, cancer, rheumatoid arthritis, selectors for the repertoire of surface peptide/MHC II complexes
Cathepsin H	Bid, Bak, BimEL, Bcl-2, Bcl-xL, Mcl-1, XIAP	Cancer
Cathepsin K	Bid, Bak, BimEL, Bcl-2, Bcl-xL, Mcl-1, XIAP	Osteoporosis, rheumatoid arthritis, osteoarthritis, pycnodysostosis
Cathepsin L	Bid, Bak, BimEL, Bcl-2, Bcl-xL, Mcl-1, XIAP, E-cadherin	Induction of apoptosis in T and B cells, cancer, thymic pathology, selectors for the repertoire of surface peptide/MHC II complexes
Cathepsin S	Bid, Bak, BimEL, Bcl-2, Bcl-xL, Mcl-1, XIAP, E-cadherin	Selectors for the repertoire of surface peptide/MHC II complexes, arthritis, bronchial asthma, psoriasis, atherosclerosis

active at neutral pH, their life-time is limited due to unfolding-induced inactivation [16,17]. Nevertheless, this life-time could be prolonged to a certain extent by acidification of the cytosol, at least in close proximity to the lysosomes, during apoptosis [18,19]. Consistent with this notion, cathepsin B, one of the most stable proteases at neutral pH, and cathepsins D and L were shown to have key roles in different models of apoptosis. For the other cathepsins, much less is known about their roles in apoptosis.

Besides their main function in lysosomal protein recycling, cathepsins were shown to be involved in a variety of physiological and pathological processes, such as maturation of the MHC class II complex, bone remodeling, keratinocyte differentiation, tumor progression and metastasis, rheumatoid arthritis and osteoarthritis, as well as atherosclerosis [20]. In contrast, their role in apoptosis was discovered more recently [21,22]. A prerequisite for their proapoptotic function is that cathepsins must be released from the lysosome into cytosol by either lysosomal destabilization [23] or lysosomal membrane permeabilization (LMP) [24]. It has been shown that while moderate lysosomal damage leads to apoptotic cell death, massive lysosomal rupture induces necrotic cell death [25,26].

A number of molecules have been found to target the lysosomal membrane. The first and probably best-characterized category includes lysosomotropic agents, such as L-leucyl-L-leucine methyl ester (Leu-Leu-OMe) [27], *O*-methyl-serine dodecylamide hydrochloride [23], sphingosine [28], 3-aminopropanal [29] and hydroxychloroquine [30]. The second category contains chemotherapeutic and anti-tumorigenic compounds that induce LMP. Among them are paclitaxel [31], etoposide [32], staurosporine [33,34], camptothecin [35], quinolone antibiotics [24], novel sigma-2 receptor ligand siramesine [36], novel compounds that target p53-independent apoptosis [37], cardenolide [38] and biphosphinic palladacycle complex [39]. Some other stimuli, such as hypochloric acid, growth factor withdrawal, bile salt, p53 protein, UV radiation, interferon-γ and cytotoxins from cobra venom were also found to be involved in LMP [40–44]. Another putative category is ROS that are generated under conditions of oxidative stress. Lysosomal destabilization has been recognized as a feature of oxidative stress-induced cell damage [45,46]. Oxidative stress may proceed directly [36,47,48] or through an amplification loop after the loss of mitochondrial outer membrane potential (MOMP) [24,30]. In addition, photosensitizers used in photodynamic therapy such as *N*-aspartyl chlorine 6 (NPe6) [49,50] and ATX-s10 [51] were found to induce LMP and release of lysosomal content. Death ligands, such as FasL, TNF-related apoptosis-inducing ligand (TRAIL) and TNF-α, were shown to induce LMP following caspase-dependent [9,21,41,52–55] or -independent [56] mechanisms. Finally, the pro-apoptotic Bcl-2 family member Bax was suggested to induce LMP by inserting in the lysosomal membrane during staurosporine-induced apoptosis [57]. A similar mechanism was proposed for free fatty acids such as palmitate [58].

While quite a lot of agents were shown to induce LMP or destabilization, only very few molecules demonstrated a stabilization property. The phosphorylated, active form of the anti-apoptotic Bcl-2 protein blocks oxidant-induced apoptosis, at least in part by stabilizing lysosomes [59,60]. It is tempting to speculate that Bcl-2 may exert its protective effect by counteracting pro-apoptotic proteins (e.g. Bax) that induce LMP. Moreover, the stress-inducible heat shock protein Hsp70 has been reported to promote cell survival by inhibiting LMP [61]. Finally, under inflammatory conditions, cytokines stabilize lysosomal membranes via an unknown mechanism, which, however, may play an important pathologic role in sepsis, chronic granulomatous disease (CGD), ulcerative colitis and appendicitis [62].

3. Cathepsins and caspase-dependent cell death pathways

The molecular mechanisms of cell death promotion by lysosomal proteases are not completely understood and may vary in a stimulus- and cell type-dependent fashion. The current concept is that lysosomal permeabilization seems to be an early event in the signalling cascade of apoptosis prior to mitochondrial membrane potential changes and release of apoptogenic factors [21,34,63]. Only a few cytosolic substrates were discovered for cathepsins (Table 1). The pro-apoptotic Bcl-2 family member Bid, initially found as a cathepsin substrate in a cell-free system [22], remains the best characterized substrate to date. Bid was confirmed as a cathepsin substrate in a variety of cell models [27,64] and it was shown *in vitro* that a number of cathepsins (B, D, L, S and K) can cleave Bid into its potent pro-apoptotic tBid (p15) fragment [27,65]. Thus, Bid could act as a mediator between lysosomes and mitochondria. This hypothesis was confirmed by the finding that incubation of mitochondria with cytosolic and lysosomal extracts severely impaired release of cytochrome c when cytosolic extract from Bid-deficient hepatocytes was used [22].

Depending on the model used, the downstream targets of Bid may vary. The most common pathway is that Bid cleavage leads to the activation of Bax, resulting in the release of apoptogenic factors, such as cytochrome c, from mitochondria (Fig. 1) [66]. This leads to an indirect activation of caspase-9 and caspase-3 via the apoptosome formation to launch the apoptotic cascade. These findings were essentially found in a cellular model in which LMP was triggered by the lysosomotropic agent L-leucyl-L-leucine methyl ester (Leu-Leu-OMe). Following mitochondrial damage, generated ROS and possibly other mitochondrial factors could feed back to the lysosome resulting in further lysosomal breakdown and exacerbation of apoptosis [67].

Another model shows that lysosomes are not the primary target of stimuli. This is the case for the death receptor pathways in which LMP was thought to be downstream of caspase-8 activation suggesting that caspases are responsible for the lysosomal breakdown and release of cathepsins into the cytosol (Fig. 1). In this context, the death ligands TNF-α [9,21], FasL [41] and TRAIL [55] were shown to induce LMP, cathepsin B or D release and Bid cleavage. In mouse embryonic fibroblasts, an alternative pathway was suggested in which caspase-8 activates caspase-9 prior to LMP in response to addition of TNF-α [52]. In this model, it was also suggested that following mitochondrial damage active caspase-9 could feed back to the

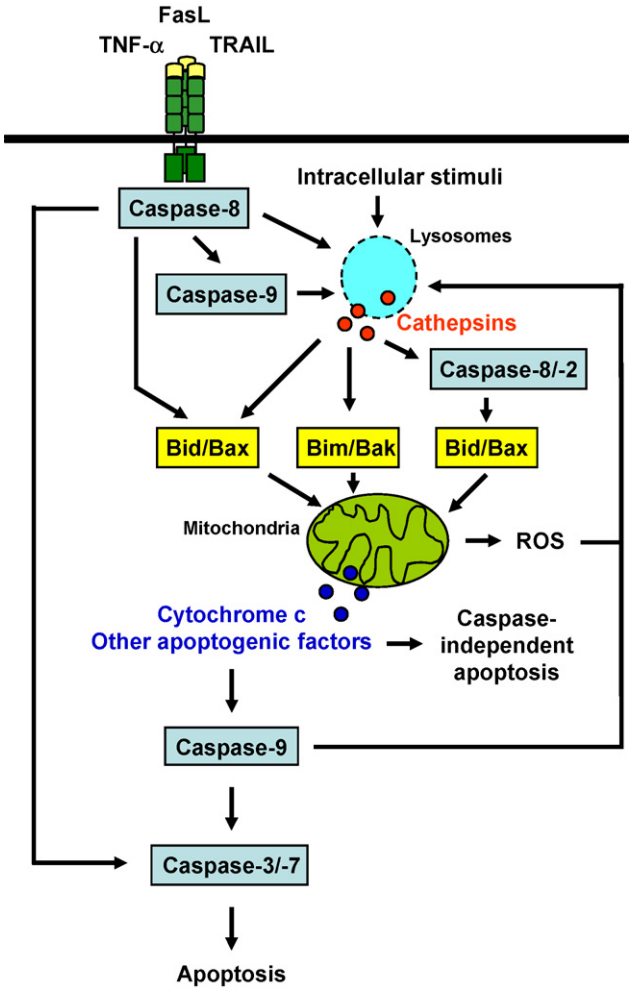

Fig. 1 – The multiple roles of cathepsins in the apoptotic signalling cascade. Depending on the cell type and the stimuli, cathepsins are rapidly released into the cytosol and are involved in two main apoptotic pathways. The first pathway includes a direct cleavage of Bid and/or Bak/Bim, translocation of these pro-apoptotic proteins to the mitochondrial outer membrane, which will induce the release of apoptogenic factors like cytochrome c, and subsequent activation of downstream caspases and apoptosis. Active caspase-9 and ROS generated by mitochondria can promote, by a positive feedback loop, further LMP to enhance apoptosis. The second pathway involves a direct cleavage of caspase-8 or -2, followed by cleavage of Bid and/or Bax, translocation of these proteins to mitochondria and similar downstream events leading to apoptosis. Moreover, the apoptogenic factor AIF is able to induce caspase-independent apoptosis under certain conditions. The exact molecular mechanisms of how cathepsins are released from lysosomes into the cytosol remain to be identified.

lysosome and enhance apoptosis. Another pathway was suggested for TNF-α-mediated hepatocyte cytotoxicity, whereby a factor associated with neutral sphingomyelinase activation (FAN) acts upstream of caspase-8/Bid, LMP and cathepsin B release into the cytosol [53]. In this pathway, procaspase-2 is activated after cathepsin B release, followed by the activation of the intrinsic pathway [54]. Moreover, camptothecin-induced apoptosis of the monocytic cells U937 also requires caspase-2 for lysosomal and mitochondrial disruption [65].

Bid is most likely not the only cellular substrate of cathepsins. When mice lacking the major intracellular inhibitor of cysteine cathepsins, stefin B, were crossed with Bid-deficient mice, no rescue of spontaneous cathepsin-dependent neuronal apoptosis was observed [68]. Similarly, Bid-deficient mouse embryonic fibroblasts are not protected against mitochondrial membrane permeabilization induced by the lysosomotropic photosensitizers ciprofloxacin and norfloxacin [24]. These findings suggest a role of additional mediators. Indeed, very recently, Droga-Mazovec et al. identified the pro-apoptotic Bcl-2 family members Bak and BimEL and the anti-apoptotic Bcl-2 family members Bcl-2, Bcl-xL and Mcl-1 as cysteine cathepsin substrates [69]. In this study, they demonstrate that lysosomal disruption triggered by Leu-Leu-OMe occurs prior to mitochondrial damage and propose that degradation of anti-apoptotic Bcl-2 family members by lysosomal cathepsins synergizes with cathepsin-mediated activation of Bid to trigger a mitochondrial pathway to apoptosis. Moreover, in this cellular model, X-linked inhibitor of apoptosis protein (XIAP) was also found to be a target of cysteine cathepsins, suggesting that cathepsins can mediate caspase-dependent apoptosis downstream of mitochondria. The requirement of Bak and/or Bax, which is another pro-apoptotic Bcl-2 family member, in lysosome-mediated apoptosis was confirmed by demonstrating that mouse embryonic fibroblasts from Bax/Bak double knockout are resistant to mitochondrial outer membrane permeabilization induced by lysosomotropic agents such as hydroxychloroquinine and norfloxacine [24], and that Bax was able to regulate LMP [57]. Finally, in cancer and especially in tumorigenesis, cathepsins were found to degrade E-cadherin, a cell adhesion molecule, which is also cleaved by caspases, arguing that secreted cathepsins may participate, together with other proteases, in tumor cell invasion [70–72].

Besides Bcl-2 family members, caspases themselves would be very suitable candidates as cathepsin substrates. Indeed, cathepsins were shown to process pro-inflammatory pro-caspase-1 and pro-caspase-11 *in vitro* [73,74]. However, both caspases have a limited, if any, role in apoptosis and it is now known that activation of pro-inflammatory caspases requires the activation platform called inflammasome [75]. In addition, pro-caspases-2, -6, -7 and -14 turned out to be weak substrates, procaspase-3 a very poor substrate and pro-caspase-12 no substrate at all for various cysteine cathepsins [22,74]. Nevertheless, a human cathepsin L-like protease and the trypanosomal cysteine cathepsin, cruzipain (which is structurally related to cathepsin L) have been shown to cleave pro-caspase-3/-7 in cell free systems and cell culture models [22,76,77]. Moreover, *in vitro* cleavage of pro-caspase-2 by cathepsin B generated a fragment showing significant mitochondrial cytochrome c-releasing activity [26]. A similar direct caspase activation by cathepsin D is occurring in neutrophils, which are crucial cells involved in innate immune responses (Fig. 1) [62]. During spontaneous neutrophil apoptosis, cathe-

psin D is rapidly released from azurophilic granules in a caspase-independent but ROS-dependent manner, leading to a direct and death receptor-independent activation of caspase-8. Therefore, direct activation of caspases by cathepsins may have physiological relevance, at least in neutrophils. Interestingly, caspase-8 has been shown to be able to activate calpain, which represents another pro-apoptotic protease in neutrophils and, perhaps, other cell types [78,79].

4. Cathepsins and caspase-independent cell death pathways

Although caspases may be indispensable for apoptosis, there is now growing evidence that programmed cell death can occur in a completely caspase-independent way [80,81]. Within a multicellular organism, caspase-independent cell death mechanisms are important protective processes for the clearance of unwanted and potentially harmful cells when caspase-dependent pathways have been totally or partially inactivated, which is often observed in cancer cells. Importantly, caspase-independent cell death can also be triggered in response to chemotherapeutic agents (e.g. staurosporine and doxorubicin) or death ligands (e.g. TNF-α) [82]. Damage of organelles including lysosomes, mitochondria and endoplasmic reticulum, leading to an increase in cytosolic calcium and ROS and the release of effector proteins (e.g. cathepsins and apoptosis-inducing factor abbreviated AIF) from organelles into the cytosol, is frequently observed in caspase-independent cell death [83].

Some models of apoptosis appear to be exclusively dependent on cathepsins [9,24,80,84]. Indeed, cathepsin D was shown to trigger activation of Bax (Fig. 1), leading to selective release of AIF from mitochondria and caspase-independent cell death of T cells [34]. This mechanism probably involves an excessive calcium influx and an overactivation of poly-ADP-ribose polymerase-1 (PARP-1) [85]. Similarly, cathepsin B is capable of executing cell death independent of the apoptotic machinery in WEHI-S fibrosarcoma and non-small cell lung cancer (NSCLC) cells [9,31]. In Jurkat T cells treated with necrotic inducers like H_2O_2 or $HgCl_2$, cathepsins B and G were shown to be released in the cytosol to cleave PARP-1 in a caspase-independent cell death pathway [86]. Furthermore, a cascade involving calpain and cathepsins has been reported, in which activated calpains induce release of lysosomal cathepsins and subsequent caspase-independent cell death in a model of ischaemic neuronal death [87].

As mentioned above, some chemotherapeutic agents also induced cathepsin-dependent but caspase-independent cell death. For instance, caspase-independent cell death, mediated by cathepsin B or D together with AIF, was seen in hepatocytes treated with camptothecin [35] as well as in fibroblasts and T cells treated with staurosporine [33,34], in several human and mice cells treated with quinolone antibiotics [24], and in NSCLC cells treated with paclitaxel [31] (Fig. 1). Finally, lysosomes are considered to be essential for autophagic cell death, which represents another form of caspase-independent programmed cell death, suggesting that lysosomal cathepsins may also play a role in this type of cell death [81,88].

5. The role of cathepsins in the immune system and the pathogenesis of diseases

Cathepsins were shown to play significant roles in physiological processes such as apoptosis, bone remodeling, keratinocyte differentiation and antigen (Ag) processing [20]. On the other hand, apoptosis is a crucial process in immunoregulation. Thus, it is likely that lysosomal cathepsins play a significant role in immune responses (Table 1). Indeed, induction and containment of immunotolerance in primary lymphoid organs and in the periphery depend on the deletion of autoreactive cell clones [12]. After organ transplantation, immunosuppressive agents such as anti-thymocyte globulins (ATG) are used to inhibit the specific immune response. In a model of high dose tolerance [89,90], Michallet et al. found that during incubation of T cells with ATG, a rapid reduction of lymphocytes proliferation and an increase in the number of apoptotic cells was depending on cytosolic cathepsin B activity. Similarly, a rapid apoptosis induction was mediated by cathepsin B and L in $CD4^+$ or $CD8^+$ T lymphocytes incubated with high Ag concentrations via CD95/CD178 interactions [91] or TNFRII/TNF interactions [92]. Same mechanisms have been shown in B cells. In addition, intracellular lysosomal and endosomal proteases of antigen presenting cells (APCs), such as dendritic cells (DCs), macrophages, B cells and other non-professional APCs, are fundamental for effective adaptive immunity. Indeed, many different lysosomal proteases (e.g. cathepsins S, L, F, V, B, D and W) and their endogenous inhibitors (such as cystatin C) have been proposed to be the major selectors for the repertoire of surface peptide-MHC II complexes and to be involved in the MHC class I pathway [93]. Thus, cathepsins are crucial modulators of adaptive immunity.

The resolution of inflammation depends on apoptosis of inflammatory cells (e.g. eosinophils and neutrophils) and their subsequent clearance by phagocytes such as macrophages, suggesting that cathepsins may be involved in innate immune responses as well. In accordance with this hypothesis, cathepsin D was shown to be a key initiator of neutrophil apoptosis by directly activating caspase-8 [62]. Importantly, this newly identified pathway of caspase-8 activation observed in neutrophils is blocked under inflammatory conditions and is crucial for the resolution of innate immune responses as demonstrated by the fact that delayed apoptosis caused by cathepsin D deficiency amplifies and prolongs neutrophilic inflammation *in vivo*. Thus, azurophilic granules, in which cathepsin D is expressed and stored, represent a new target for the development of anti-inflammatory drugs. Moreover, Blomgran et al. reported that during microbe-induced apoptosis of human neutrophils, ROS-dependent release of cathepsin B induces the cleavage of Bid, mitochondrial damage and subsequent caspase activation and apoptosis [64]. Together and depending on the model, these studies confirm that some cathepsins are important modulators of innate immune responses.

Cathepsins can be detrimental to cells and tissues if they are not properly kept under control. For instance, due to altered expression (e.g. upregulation in cancer and metastasis progression), proteolytic activity (unbalanced amount between proteases and endogenous inhibitors) and localization (increased secretion outside the cells), they are often

involved in pathologies [94]. Deregulated cathepsins activity is therefore thought to be a cause or contributing factor for diseases such as cancer, bronchial asthma, atherosclerosis, Alzheimer's disease, periodontitis, rheumatoid arthritis, inflammatory bowel disease, osteoarthritis, and pulmonary fibrosis underlying the biological importance of these lysosomal proteases. On the other hand, lysosomes may be targets for anti-tumor therapy [95]. For instance, agents, which are able to induce LMP, provoke cathepsin-mediated cytotoxicity (e.g. in tumor cells) [37].

Finally, hereditary deficiencies in lysosomal enzymes might be pathogenic because of an increase in apoptosis. Farber's disease, for example, a disorder caused by ceramidase deficiency, results in an accumulation of ceramide, a pro-apoptotic second messenger reported to induce LMP and to inhibit the respiratory chain which in turn induces MOMP [96]. Moreover, loss-of-function mutations of cystatin B, a cytosolic inhibitor of lysosomal cysteine cathepsins, cause Unverricht-Lundborg disease, an autosomal recessive inherited form of epilepsy [97]. Similarly, cystatin-B$^{-/-}$ mice manifest signs of apoptosis affecting cerebellar granule cells [98].

6. Conclusions

Apoptosis is a crucial physiological process that allows the controlled removal of old, unwanted or potentially harmful cells from an organism. It is hence not surprising that defects in the apoptotic mechanism are closely linked to the development of various diseases. For many years, most of the studies focussed on the role of caspases in apoptosis. Although these cysteine proteases play a very central role in apoptotic signalling mechanisms, there is growing evidence that other proteases contribute to the regulation of apoptosis, in particular lysosomal cathepsins. Knockout mice lacking single cathepsin family members develop normally and do not display abnormal phenotypes at the time of birth. However, these mice can develop abnormalities later in life (such as cathepsin D knockout mice), suggesting that cathepsins play an important role in postnatal tissue homeostasis and pathophysiology.

Depending on the stimuli and the model used, cathepsins are released in a very early event and are capable of triggering activation of specific substrates, such as caspase-8 and pro-apoptotic Bcl-2 family members, mitochondrial dysfunction with subsequent caspase activation and cellular demise. A variety of different models confirm the biological importance of cathepsins in the regulation of apoptosis under physiological conditions. Importantly, lysosomal cathepsins were shown to be key regulators of the life span of inflammatory cells like neutrophils, T cells, and B cells, confirming their physiological relevance in immune responses.

Understanding the regulatory role of cathepsins in the immune system will provide us with important information on how to efficiently modulate immune responses to treat diseases. Further investigation regarding the mechanism(s) of their release from lysosomes and cytosolic cathepsin substrates are required. These findings will help to design novel therapeutic strategies to prevent cathepsin-related damage in pathological processes, such as neurodegeneration and autoimmune diseases or to stimulate apoptosis through LMP and subsequent release of cathepsins in processes such as cancer and inflammatory diseases.

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