Yeast as a tool to explore cathepsin D function

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ABSTRACT Cathepsin D has garnered increased attention in recent years, mainly since it has been associated with several human pathologies. In particular, cathepsin D is often overexpressed and hyperscreted in cancer cells, implying it may constitute a therapeutic target. However, cathepsin D can have both anti- and pro-survival functions depending on its proteolytic activity, cellular context and stress stimulus. Therefore, a more detailed understanding of cathepsin D regulation and how to modulate its apoptotic functions is clearly needed. In this review, we provide an overview of the role of cathepsin D in physiological and pathological scenarios. We then focus on the opposing functions of cathepsin D in apoptosis, particularly relevant in cancer research. Emphasis is given to the role of the yeast protease Pep4p, the vacuolar counterpart of cathepsin D, in life and death. Finally, we discuss how insights from yeast cathepsin D and its role in regulated cell death can unveil novel functions of mammalian cathepsin D in apoptosis and cancer.

CATHEPSINS Cathepsins are members of a large protease family, which can be subdivided according to their structure and active-site amino acid into cysteine (cathepsins B, C, F, H, K, L, O, S, V, W, and X), serine (cathepsins A and G), and aspartic cathepsins (cathepsins D and E). While cathepsins B, L, H, C and D are ubiquitously expressed in human tissues, expression of cathepsins A, G, K, S, V, X and W is tissue and cell type specific [1-4]. In general, cathepsins are found in acidic cellular organelles, lysosomes and endosomes. Initially, their function was thought to be limited to bulk degradation of proteins delivered to the lysosome by endocytosis or autophagocytosis. However, it was later demonstrated that cathepsins possess highly specific and directed proteolytic activity, and that they can be found in other cellular compartments [5-10]. Numerous physiological functions of cathepsins have been uncovered, including a role in hormone and antigen processing, bone and tissue remodeling, growth factor and proenzyme activation and, more recently, in the immune response [5, 6, 11-13]. Cathepsins also participate in apoptosis and are translocated from the lysosomal lumen to the cytosol of mammalian cells through lysosomal membrane permeabilization (LMP) in response to a variety of apoptotic signals [14-16]. These lysosomal proteases can also be secreted from the cell and degrade extracellular matrix proteins such as collagen, fibronectin, proteoglycans and laminin [17]. In addition to their physiological function, cathepsins have also been associated with several pathologies such as cardiovascular diseases, osteoporosis, rheumatoid arthritis, atherosclerosis and cancer [6, 11, 17-19]. Elucidating the mechanisms underlying the involvement of cathepsins in the pathogenesis of these diseases, and how they can be modulated to develop new prevention and therapeutic strategies, has therefore taken center stage. Among cathepsins, cathepsin D (CatD) has attracted increased attention in recent years due to its importance in the mediation of lysosomal cell death pathways and in cancer. In this review, we will concentrate on both physiological and pathological functions of CatD, as well as on yeast as a model system to study CatD pathophysiology.

ROLE OF CATHEPSIN D IN CELLULAR PHYSIOLOGY AND PATHOLOGY CatD is a soluble aspartic endopeptidase found in the lysosomes of most mammalian cells. Like other cathepsins,
CatD is activated by proteolytic cleavage of the synthetized inactivezymogen (proCatD), which is composed of anN-terminal signal peptide, a propeptide, and a catalyticdomain [20-22]. The signal peptide directs the nascentchain to the endoplasmatic reticulum, where it is cleavedinthelumen. ProCatD is then N-glycosylated and transportedto the Golgi, where the N-glycan structures acquire man nose-6-phosphate (Man-6P) residues that can bind to Man 6P receptor(s) (Man-6PR), and the complex is directed tothe lysosomal compartment [23]. In the acidic milieu, proCatD (52 kDa) undergoes further proteolytic processing bycleavage of the proregion, resulting in the 48 kDa singlechain intermediate active form. Finally, this chain is processed into mature active CatD, composed of heavy (34kDa) and light (14 kDa) chains linked by non-covalentinteractions [24-26]. It has been shown that CatD processinginvolves cysteine cathepsins [26, 27] and, more recently,that it is independent of its own catalytic function and au to-activation but requires CatL and CatB [28]. Although proCatD and CatD are mostly intracellular, they can also localizewithin the extracellular matrix and synovial fluid of cartilage [29-31]. ProCatD/CatD are also found in human,bovine and rat milk [32-34], serum, sweat and urine [35,36], and extracellularly in macrophage-rich regions of atherosclerotic lesions [37]. ProCatD secretion by human keranocytes [38], mammalian epithelial cells [39] and different types of cancer cells [18, 40] was also demonstrated. It is widely accepted that the major function of CatD is its involvement in general protein degradation and turn over within the lysosomal compartment. However, CatD hasalso emerged as an important regulator and signaling mol ecule with numerous physiological functions. These includeactivation of enzymatic precursors, prohormones andgrowth factors, processing of brain-specific antigens, tissuehomeostasis, and participation in apoptosis [18, 41]. CatD hasalso been associated with different pathological scenarios such as cancer progression and metastasis, Alzheimer’s disease, atherosclerosis and inflammatory disorders [11, 12, 40, 42], and found to be a specific biomarker for several pathologies. The involvement of CatD in bothphysiological and pathological processes has been ad dressed in multiple studies, some of which are summarized in Table 1 [38, 43-62]. A more detailed description of therole of CatD in cancer is given below.

### THE ROLE OF CATHEPSIN D IN CANCER

Numerous reports have demonstrated that CatD is overex pressed in several cancer types [18, 40, 42, 63-65], oftencorrelating with poor prognosis. In particular, CatD is con sidered an independent prognostic marker in breast cancer associated with metastatic risk [66-68] and in colorectal cancer (CRC) [69, 70]. Mechanistically, the majority of reports attribute its role in cancer to overexpression of proCatD. As an example, transfection of rat tumor cells with human proCatD cDNA leads to increased proliferation, invasion and metastasis in vitro and in vivo [71]. Accordingly, anti-proCatD antibodies can inhibit tumor growth both in vitro and in vivo [72-74]. Overexpressed proCatD escapes normal targeting routes and is hypersecreted to the extracellular milieu, where it can act in multiple fashions. On

### TABLE 1. Cellular roles of cathepsin D in physiological and pathological processes.

| Role | Model | References |
|------|-------|------------|
| Limited proteolysis of proteins regulating cell growth and/or tissue homeostasis | In vivo: Cat D-deficient mice | [43] |
| Postnatal tissue homeostasis including tissue renewal, remodeling, aging and RCD | In vivo: Cat D-deficient mice; Cat D-mutant mice | [44-47] |
| Neuronal ceroid lipofuscinosis in both animals and humans characterized by severe neurodegeneration, developmental regression, visual loss and epilepsy | In vivo: Cat D-deficient mice; Cat D-mutant mice Human pathologies Animal diseases | [45, 48-51] |
| Wound healing, epidermal differentiation and pathological conditions such as psoriasis | In vivo: Cat D-deficient mice In vitro: normal and psoriatic keranocytes Human patients | [52-54] |
| Proliferation and regeneration in keranocytes and possibly in skin regeneration | In vitro: keranocyte cell line HaCaT | [38] |
| Processing of proteins involved in Alzheimer disease pathogenesis, such as apolipoprotein E (apoE) and Tau protein | Human patients Recombinant protein | [55, 56] |
| Post-partum cardiomyopathy resulting in heart failure | In vivo: mutant mice | [57] |
| Autism pathogenesis | Autistic subjects | [58] |
| Innate immune responses and Parkinson disease | In vivo: Cat D-deficient mice Human patients | [59, 60] |
| Intracellular metabolism, transport of phospholipids and cholesterol | Human patients | [61] |
| Atherosclerotic lesions associated with proCatD release from monocyte-derived macrophages | Atherosclerosis patients In vitro: cultured atherosclerotic plaques | [62] |
one hand, it can exert an autocrine effect, inducing cancer cell growth by interacting with cell surface receptors [72, 75-77]. This autocrine role has so far been observed in breast, prostate, ovarian and lung cancer cells [72-74, 78]. In addition, proCatD can play a crucial paracrine role in the tumor microenvironment by stimulating fibroblast outgrowth and tumor angiogenesis [71, 79], as well as inhibiting anti-tumor responses [80]. When in the tumor microenvironment, proCatD may also affect stromal cell behavior and/or degrade components from the extracellular matrix [81, 82], including the release of growth factors [83]. Although it has been suggested that proCatD can be processed in the acidic extracellular space to catalytically active CatD [84], the enzymatic activity of CatD is reportedly not required for its mitogenic role. Indeed, a proteolytically inactive mutant of CatD (D231N) is still mitogenic for fibroblasts [85], as well as for cancer cells both in vitro, in three-dimensional matrices, and in athymic nude mice [71, 86]. Similarly, proCatD stimulates angiogenesis in tumor xenografts of athymic nude mice independently of its catalytic activity [85], also suggesting that CatD can signal through protein-protein interactions.

Though less extensive, there are also examples of CatD roles in cancer cells that are not attributed to proCatD. For instance, intracellular CatD can stimulate cancer cell growth by inactivating secreted growth inhibitors [87, 88]. Moreover, mature CatD released into the cytosol as a consequence of the reportedly higher susceptibility of cancer cells to LMP [15, 89] may interact with and/or degrade pro- and anti-apoptotic proteins, modulating cell death [41].

Targeting CatD is a promising strategy in the clinic, but requires further detailed elucidation of its mechanisms of action. In the following section, we focus on the role of CatD in the apoptotic process, which is of particular relevance for cancer research. These studies may however also offer clues into the function of CatD in other physiological and pathological scenarios.

**OPPOSING FUNCTIONS OF CATHEPSIN D IN APOPTOSIS**

In recent years, multiple studies have shown that CatD is a central player in the apoptotic response, both under physiological and pathological conditions. In fact, depending on the cell type and context, CatD can induce or inhibit apoptosis, acting through different mechanisms [41]. On one hand, CatD can directly induce apoptosis triggered by several stimuli such as staurosporine [90], etoposide, 5-fluorouracil and cisplatin [91], as well as resveratrol [92] and others, possibly mediated by intrinsic or extrinsic pathways [41]. In the intrinsic pathway, the role of CatD is linked to the release of mature 34 kDa CatD into the cytosol and cleavage of Bid to form tBid, triggering insertion of the pro-apoptotic protein Bax into the mitochondrial membrane [15]. Subsequent mitochondrial outer membrane permeabilization leads to the release of pro-apoptotic molecules such as cytochrome c and apoptosis inducing factor (AIF) to the cytosol [15]. For instance, it has been shown that CatD mediates cytochrome c release and caspase activation in human fibroblasts undergoing staurosporine-induced apoptosis [90], and cleaves Bid and promotes apoptosis via oxidative stress-induced LMP in human neutrophils [93]. In addition, Pepstatin A and/or knockdown of CatD expression by RNA interference prevents resveratrol toxicity, impeding Bax oligomerization, mitochondrial membrane permeabilization, cytochrome c release and caspase 3 activation in DLD1 and HT29 CRC cell lines [92]. One study also reports that CatD mediates selective release of AIF in T lymphocytes entering the apoptosis early commitment phase through activation of Bax in a Bid-independent manner [94]. This shows that CatD can be involved in caspase-independent apoptosis by activating Bax independently of Bid cleavage. Other studies strongly suggest that cytosolic CatD may have an additional role involving protein-protein interactions. As examples, it has been shown that overexpression of either catalytically active or inactive CatD by cancer cells enhances apoptosis-dependent chemo-sensitivity [95], and that stress-induced apoptosis is not affected in fibroblasts synthesizing a catalytically inactive CatD [96]. Additionally, microinjection of inactive proCatD into the cytosol of both human fibroblasts and HeLa cells induces apoptosis [97]. Interestingly, one report also indicates that cytosolic mature CatD may reach the nucleus during cell death [98].

In contrast with the multiple studies showing CatD is pro-apoptotic, other studies describe an anti-apoptotic function of CatD. Most of these suggest it plays an anti-apoptotic role in cancer cells. For example, CatD down-regulation sensitizes human neuroblastoma cells to doxorubicin-induced apoptosis, while CatD overexpression has the opposite effect [99]. Accordingly, inhibition of CatD with pepstatin A induces caspase-dependent apoptosis in neuroblastoma cell lines [100]. Moreover, overexpression of intracellular CatD in mouse xenografts using rat-derived cell lines inhibits apoptosis [71], and expression of wild type or a catalytic mutant of CatD promotes survival and invasive growth of CatD-deficient fibroblasts [85]. Another study in glioblastoma cells proposes that CatD stimulates autophagy induction, inhibiting apoptotic cell death under genotoxic conditions [101]. More recently, we showed that inhibition of CatD in CRC cells with small interfering RNA (siRNA) or pepstatin A enhances acetate-induced apoptosis, associated with a decrease in mitochondria degradation independently of autophagy [102, 103]. An anti-apoptotic role of CatD has also been described under physiological conditions using CatD-deficient mice [43-45]. Indeed, mutant mice developed apoptosis in the thymus, thalamus and retina.

In summary, it is well documented that CatD plays an important role in apoptosis regulation, both with and without involvement of its proteolytic activity. However, the exact role of CatD in apoptosis, particularly what determines whether this protease plays an anti- or pro-apoptotic function remains poorly understood. In this regard, a simpler model system would be particularly useful to offer additional clues into this dichotomy.
YEAST VACUOLAR PROTEASES

The versatility of the yeast *Saccharomyces cerevisiae* to study several conserved cellular functions such as cell metabolism, cell cycle, cell death and organelle biogenesis has justified the attractiveness of this system to study more complex mammalian physiological and pathological processes [104-108]. Like other organelles, the yeast vacuole is functionally similar to its higher eukaryote counterpart, the lysosome. It harbors seven characterized proteases, namely three aminopeptidases, three serine proteases and one aspartyl protease. Among these, two are endopeptidases: proteinase A (Pep4p), ortholog to human CatD, and protease B (Prb1p). Five are exopeptidases: carboxypeptidase Y (CPY), ortholog to human CatA, carboxypeptidase S (CP51), aminopeptidase I (Ape1) and Y (Ape3), and dipeptidylaminopeptidase B (Dap2).

More recently, Hecht *et al.* reported an eighth vacuolar protease, a transmembrane metalloprotease (Pff1) [109], but although evidence of Pff1 vacuolar localization was shown, its proteolytic activity has yet to be demonstrated.

The endopeptidases are responsible for the majority of bulk protein degradation, including of plasma membrane proteins. They are also fundamental for activation of the vacuolar proteolytic cascade, particularly Pep4p, since it is involved in proteolytic activation of Prb1p, CPY and Ape1 [110, 111]. Prb1p, in turn, participates in the activation of Pep4p, CPY, CPS1, Ape1 and Ape3. Both carboxypeptidases and Ape1 are involved in peptide and glutathione degradation, respectively, but are not required for zymogen activation [111, 112].

Substrates for the vacuolar proteases are mostly imported via endocytosis (extracellular and cell surface proteins) or autophagy (cytoplasmic material and organelles). Autophagy is activated under nutrient deprivation conditions, and both Pep4p and Prb1p are implicated in the dissolution of autophagic bodies [113, 114].

In addition, vacuolar proteases play a role in sporulation. While absence of Prb1p activity alone results in partial reduction of sporulation, absence of Prb1p activity in a mutant lacking both CPY and CPS1 leads to almost complete loss of sporulation ability [115]. In addition to ensuring protein homeostasis under physiological conditions, vacuolar proteolysis therefore also appears to be a stress-responsive process, particularly under nutrient stress conditions and during sporulation. However, additional roles for vacuolar proteases have emerged in recent years, in particular for Pep4p.

**Pep4p PROTEASE - THE YEAST CATHEPSIN D**

Yeast CatD (Pep4p), like its lysosomal counterpart, is synthesized as an inactive zymogen, traveling via the endoplasmic reticulum and Golgi to the acidic vacuoles, where it is activated through proteolytic removal of the inhibitory propeptide [116]. Although Pep4p is mainly located in the vacuole, different cell death stimuli can lead to its release to the cytosol, involving a selective vacuolar membrane permeabilization (VMP) typical of apoptotic death.

Mason *et al.* were the first to report that Pep4p translocates from the vacuole to the cytosol [117]. These authors observed an increase in nuclear permeability associated with increased accumulation of reactive oxygen species (ROS) during H$_2$O$_2$-induced cell death, and found that Pep4p is released into the cytosol and degrades nucleoporins during this process. However, Pep4p did not affect resistance to H$_2$O$_2$-induced cell death, probably because it migrates out of vacuoles after cells are effectively unviable. They further showed that the release of a Pep4p-EGFP (Enhanced Green Fluorescent Protein) fusion from the vacuole in H$_2$O$_2$-treated cells was not associated with major rupture of the vacuolar membrane, as cells maintained a vacuolar lumen morphologically distinct from the cytosol. Other authors reported that Pep4p is involved in protein degradation and removal of oxidized proteins during H$_2$O$_2$-induced oxidative stress, but also did not ascribe a role for this protease in cell death induced by H$_2$O$_2$ [118].

Another study showed that stabilization of the actin cytoskeleton caused by lack of the actin regulatory protein End3p leads to loss of mitochondrial membrane potential, accumulation of ROS, increase in VMP and consequent migration of Pep4p to the cytosol, as well as apoptotic cell death [119]. In that study, Pep4p-EGFP was visualized exclusively in the vacuole lumen in wild type cells, but distributed throughout the entire cell in an *END3*-deficient strain. Again, no role was attributed to this protease in actin-stabilized dying cells.

Pep4p is also involved in programmed nuclear destruction during yeast gametogenesis [120]. Using cells co-expressing Pep4p-mCherry and Vma1-GFP, a GFP-tagged vacuolar membrane protein, Pep4p was shown to translocate from the vacuole into the ascal compartment of early postmeiotic cells during sporulation, with preservation of vacuolar integrity.

These observations show that VMP seems to mimic LMP in human cells. However, they do not indicate whether yeast vacuolar proteases play a role in cell survival and regulated death.

In this regard, it has been shown that Pep4p has a pro-survival role during chronological aging, since a Pep4p-deficient mutant has a shortened lifespan associated with higher levels of carbonylated proteins [118]. Carmona-Gutiérrez *et al.* further showed that deletion of *PEP4* results in both apoptotic and necrotic cell death during chronological aging [121]. Using a panel of Pep4p mutants, they conclude that Pep4p plays a dual pro-survival role composed of both anti-apoptotic and anti-necrotic functions, conferred by its proteolytic activity and its proteolytically inactive propeptide, respectively. We also previously found that Pep4p-EGFP translocates to the cytosol during acetic acid-induced apoptosis involving selective VMP in *S. cerevisiae* W303 cells, with preservation of both vacuolar and plasma membrane integrity [122]. Moreover, we demonstrated that Pep4p is required for increased cell survival and for efficient autophagy-independent mitochondrial degradation in response to this acid in a manner depending on its catalytic activity [122, 123]. This suggests that VMP associated with Pep4p release may act as an...
alternative mitochondrial degradation process, delaying cell death. In contrast, we recently demonstrated that absence of \textit{PEP4} resulted in increased resistance to acetic acid in \textit{S. cerevisiae} BY4741 cells [124]. This prompted the hypothesis that Pep4p plays a dual function in acetic acid-induced cell death depending on the genetic background, providing an interesting tool to explore the molecular determinants of CatD function.

**YEAST AS A TOOL TO EXPLORE THE ROLE OF CATHEPSIN D IN APOPTOSIS AND CANCER**

It is widely established that the process of regulated cell death (RCD) involves a genetically encoded molecular machinery [125]. Core components of this machinery are conserved in yeast, which can undergo RCD exhibiting typical markers of apoptosis, autophagy and necrosis [126-128]. Thus, this eukaryotic organism has been used extensively to study the molecular mechanisms of RCD pathways, reviewed elsewhere [126-129]. These studies encompass not only analysis of yeast endogenous death pathways but also heterologous expression of human proteins involved in apoptosis, such as caspases, Bcl-2 family proteins, PKC isoforms and the p53 tumor suppressor protein [130, 131].

As discussed above, the role of the lysosome-like vacuole in the regulation of RCD has been investigated in yeast, where it has been shown to play a role similar to lysosomes [132, 133]. However, the use of this model organism to study lysosomal cell death pathways in general and cathepsin function in particular is still underexplored. So far, only translocation of Pep4p to the cytosol during yeast apoptosis has been clearly demonstrated by different authors [117, 119, 122]. One other study shows that the RNase T2 family member Rny1p is also released from the vacuole into the cytosol during oxidative stress, with preservation of vacuolar membrane integrity, directly promoting cell death [134]. The need for a comprehensive analysis of the VMP process and the vacuolar proteins released in response to different stimuli is therefore evident.

Another approach that has not been sufficiently exploited is the heterologous expression of cathepsins in yeast. Two studies have shown that rat cathepsin L and D precursor polypeptides are recognized by mechanisms similar to those involved in the intracellular sorting of vacuolar proteins in yeast cells [135, 136]. We therefore sought to further explore this tool to understand the function of human CatD. As mentioned above, we previously

![FIGURE 1: Survival of \textit{S. cerevisiae} cells expressing Bax during acetic acid treatment.](image)
showed the parallel between the role of human and yeast CatD in acetic acid-induced apoptosis and in the degradation of damaged mitochondria, which render CRC/yeast cells more resistant to apoptosis induced by acetic acid [102, 122]. We now found that heterologous expression of human CatD in yeast PEP4-deficient cells reverts their sensitivity to acetic acid-induced apoptosis and delays mitochondrial degradation [103], as previously observed for wild type Pep4p [122, 123]. These results provide evidence that the role of CatD in both apoptosis and mitochondrial degradation is conserved through evolution. Further elucidation of the molecular mechanisms underlying the involvement of CatD in apoptosis and in mitochondrial degradation will now be crucial to develop novel strategies to specifically inhibit this protease in apoptosis deficiency-associated diseases, such as cancer.

Taking into account the multiple functions of CatD, one caveat of using CatD inhibitors could be a negative effect on Bax activation, release of cytochrome c and downstream caspase activation. To address this question, we exploited the well-established system of heterologous expression of Bax in yeast, which lacks obvious orthologs of the Bcl-2 family, and allows studying how absence of yeast CatD affects Bax activity without interference from other Bcl-2 family members. Using yeast cells heterologously expressing a cytosolic inactive form of human Bax, which was activated by exposure to acetic acid, we could discard this hypothesis since absence of Pep4p enhanced Bax-induced cell death (Figure 1). It will be interesting to further exploit this system with heterologous co-expression of Bax and human CatD, in order to dissect the role of this lysosomal protease in the regulation of Bax activity independently of Bid.

As a final conclusion, it becomes apparent that the approaches with yeast have already provided and can further offer new perspectives for an increased understanding of the role of CatD in mammalian apoptosis, and its implications in cancer. Indeed, studies with yeast further reinforce the use of this eukaryotic organism as a valuable model to identify and characterize novel RCD processes, and open the door to new clinical opportunities, with a substantial impact in public health.

ACKNOWLEDGMENTS

This work was supported by FEDER through POFC – COMPETE and by Fundação para a Ciência e Tecnologia through projects PEst-OE/BIA/UI4050/2014 and FCTANR/BEX-BCM/0175/2012, as well as fellowships to H. Pereira (SFRH/BD/73139/2010), C.S.F. Oliveira (SFRH/BD/77449/2011), L. Castro (SFRH/BD/93589/2013) and S. Chaves (SFRH/BPD/89980/2012).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Please cite this article as: H. Pereira, C.S.F. Oliveira, L. Castro, A. Preto, S. R. Chaves, M. Córte-Real (2015). Yeast as a tool to explore cathepsin D function. Microbial Cell 2(7): 225-234. doi: 10.15698/mic2015.07.212

REFERENCES

1. Lecaille F, Kaleta J, and Brömme D (2002). Human and Parasitic Papain-Like Cysteine Proteases: Their Role in Physiology and Pathology and Recent Developments in Inhibitor Design. Chem Rev 102(12): 4459-4488.

2. Turk B, Turk D, and Turk V (2000). Lysosomal cysteine proteases: more than scavengers. Biochim Biophys Acta 1477(1-2): 98–111.

3. Wex T, Levy B, Smeekens SP, Ansorge S, Desnick RJ, and Bromme D (1998). Genomic structure, chromosomal localization, and expression of human cathepsin W. Biochem Biophys Res Commun 248(2): 255–261.

4. Brömme D, Li Z, Barnes M, and Mehler E (1999). Human cathepsin V functional expression, tissue distribution, electrostatic surface potential, enzymatic characterization, and chromosomal localization. Biochemistry (Moscow) 38(8): 2377–2385.

5. Brix K, Dunkhorst A, Mayer K, and Jordans S (2008). Cysteine cathepsins: cellular roadmap to different functions. Biochimie 90(2): 194–207.

6. Turk V, Stoka V, Vasiljeva O, Renko M, Sun T, Turk B, and Turk D (2012). Cysteine cathepsins: from structure, function and regulation to new frontiers. Biochim Biophys Acta 1824(1): 68–88.

7. Pratt MR, Sekedat MD, Chiang KP, and Muir TW (2009). Direct measurement of cathepsin B activity in the cytosol of apoptotic cells by an activity-based probe. Chem Biol 16(9): 1001–1012.

8. Hook V, Funkelstein L, Wegrzyn J, Bark S, Kindy M, and Hook G (2012). Cysteine Cathepsins in the secretory vesicle produce active peptides: Cathepsin L generates peptide neurotransmitters and cathepsin B produces beta-amyloid of Alzheimer’s disease. Proteolysis 50 Years Discov Lysosome 1824(1): 89–104.

9. Goulet B, Baruch A, Moon N-S, Poirier M, Sansregret LL, Erickson A, Bogyo M, and Nepveu A (2004). A cathepsin L isoform that is devoid of a signal peptide localizes to the nucleus in S phase and processes the CDP/Cux transcription factor. Mol Cell 14(2): 207–219.

10. Duncan EM, Muratore-Schroeder TL, Cook RG, Garcia BA, Shanabowitz J, Hunt DF, and Allis CD (2008). Cathepsin L proteolytically processes histone H3 during mouse embryonic stem cell differentiation. Cell 135(2): 284–294.

11. Reiser J, Adair B, and Reinheckel T (2010). Specialized roles for cysteine cathepsins in health and disease. J Clin Invest 120(10): 3421–3431.

12. Conus S and Simon H-U (2010). Cathepsins and their involvement in immune responses. Swiss Med Wkly 140: w13042.
13. Jacobson LS, Lima H, Goldberg MF, Gocheva V, Tsiperson V, Sutterwala FS, Joyce JA, Gapp BV, Blomen VA, Chandran K, Brummelkamp TR, Diaz-Griffero F, and Brojak J (2013). Cathepsin-mediated necrosis controls the adaptive immune response by Th2 (T helper type 2)-associated adjuvants. *J Biol Chem* 288(11): 7481–7491.

14. Repnik U, Hafner Česen M, and Turk B (2014). Lysosomal membrane permeabilization in cell death: Concepts and challenges. *Mitochondrion* 19 Pt A: 49–57.

15. Boya P and Kroemer G (2008). Lysosomal membrane permeabilization in cell death. *Oncogene* 27(50): 6434–6451.

16. Česen MH, Pegan K, Spes A, and Turk B (2012). Lysosomal pathways to cell death and their therapeutic applications. *Exp Cell Res* 318(11): 1245–1251.

17. Fonović M and Turk B (2014). Cysteine cathepsins and extracellular matrix degradation. *Biochim Biophys Acta* 1840(8): 2560–2650.

18. Benes P, Vetvicka V, and Fusek M (2008). Cathepsin D—many functions of one aspartic protease. *Crit Rev Oncol Hematol* 68(1): 12–28.

19. Saftig P, Hunziker W, Moritz JD, Schu P, and von Figura K (1998). Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. *Proc Natl Acad Sci U S A* 95(23): 13453–13458.

20. Hasilik A and Neufeld EF (1980). Biosynthesis of lysosomal enzymes in fibroblasts. Synthesis as precursors of higher molecular weight. *J Biol Chem* 255(10): 4937–4945.

21. Erickson AH (1989). Biosynthesis of lysosomal endopeptidases. *J Cell Biochem* 40(1): 31–41.

22. Gieselmann V, Pohlmann R, Hasilik A, and von Figura K (1983). Biosynthesis and transport of cathepsin D in cultured human fibroblasts. *J Cell Biol* 97(1): 1–5.

23. Baranski TJ, Koelsch G, Hartsuck JA, and Kornfeld S (1991). Mapping and molecular modeling of a recognition domain for lysosomal enzyme targeting. *J Biol Chem* 266(34): 23365–23372.

24. Erickson AH, Conner GE, and Blobel G (1981). Biosynthesis of a lysosomal enzyme. Partial structure of two transient and functionally distinct NH2-terminal sequences in cathepsin D. *J Biol Chem* 256(21): 11224–11231.

25. Conner GE and Richo G (1992). Isolation and characterization of a stable activation intermediate of the lysosomal aspartyl protease cathepsin D. *Biochemistry (Moscow)* 31(4): 1142–1147.

26. Gieselmann V, Hasilik A, and von Figura K (1985). Processing of human cathepsin D in lysosomes in vitro. *J Biol Chem* 260(5): 3215–3220.

27. Samarel AM, Ferguson AG, Decker RS, and Lesch M (1989). Effects of cysteine protease inhibitors on rabbit cathepsin D maturation. *Am J Physiol* 257(6 Pt 1): C1069–C1079.

28. Laurent-Matha M, Vercruysse C, Ghigo M, and Vetvicka V (2004). Cathepsins mediate tumor metastasis. *World J Biol Chem* 4(4): 91–101.

29. Poole AR, Hembry RM, and Dingle JT (1974). Cathepsin D in cartilage: the immunohistochemical demonstration of extracellular enzyme in normal and pathological conditions. *J Cell Sci* 14(1): 139–161.

30. Bille A and Osterlin S (1976). Cathepsin D activity in bovine articular cartilage, synovial membrane and fluid: degradation of cartilage proteoglycans from same joint. *J Rheumatol* 3(4): 400–408.

31. Vittorio N, Crissman JD, Hopson CN, and Herman JH (1986). Histologic assessment of cathepsin D in osteoarthritic cartilage. *Clin Exp Rheumatol* 4(3): 221–230.

32. Větvicka V, Vágner J, Baudys M, Tang J, Foundling SI, and Fusek M (1993). Human breast milk contains procathepsin D–detection by specific antibodies. *Biochem Mol Biol Int* 30(5): 921–928.

33. Larsen LB and Petersen TE (1995). Identification of five molecular forms of cathepsin D in bovine milk. *Adv Exp Med Biol* 362: 279–283.

34. Benes P, Koelsch G, Dvorak B, Fusek M, and Vetvicka V (2002). Detection of procathepsin D in rat milk. *Comp Biochem Physiol B Biochem Mol Biol* 133(1): 113–118.

35. Zühlisdofer M, Imort M, Hasilik A, and von Figura K (1983). Molecular forms of beta-hexosaminidase and cathepsin D in serum and urine of healthy subjects and patients with elevated activity of lysosomal enzymes. *Biochem J* 213(3): 733–740.

36. Baechle D, Flad T, Cansier A, Schütte B, Tolson J, Herrmann T, Dihazi H, Beck A, Mueller GA, Mueller M, Stevanovic S, Garbe C, Mueller CA, and Kaibacher H (2006). Cathepsin D is present in human eccrine sweat and involved in the postsecretory processing of the antimicrobial peptide DCD-1L. *J Biol Chem* 281(9): 5406–5415.

37. Hakala JK, Oksjoki R, Laine P, Du H, Grabowski GA, Kovanen PT, and Pentikäinen MO (2003). Lysosomal enzymes are released from cultured human macrophages, hydrolyze LDL in vitro, and are present extracellularly in human atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 23(8): 1430–1436.

38. Vashistha A, Sarawat Ohri S, Vetvickova J, Fusek M, Ulrichova J, and Vetvicka V (2007). Procathepsin D secreted by HaCaT keratinocyte cells - A novel regulator of keratinocyte growth. *Eur J Cell Biol* 86(6): 303–313.

39. Lhinder M, Castino R, Bougueny E, Isidoro C, and Ollivier-Bousquet M (2004). Cathepsin D released by lactating rat mammary epithelial cells is involved in prolactin cleavage under physiological conditions. *J Cell Sci* 117(Pt 51): 5155–5164.

40. Masson O, Bach A-S, Deroce D, Prébois C, Laurent-Matha V, Pattingre S, and Liaudet-Coopman E (2010). Pathophysiological functions of cathepsin D: Targeting its catalytic activity versus its protein binding activity? *Biochimie* 92(11): 1635–1643.

41. Minarowska A, Minarowski L, Karwowska A, and Gacko M (2007). Regulatory role of cathepsin D in apoptosis. *Pol Acad Sci Pol Histochem Cytochem Soc* 45(3): 159–163.

42. Tan G-J, Peng Z-K, Lu J-P, and Tang F-Q (2013). Cathepsins mediate tumor metastasis. *World J Biol Chem* 4(1): 91–101.

43. Saftig P, Hetman M, Schmahl W, Weber K, Heine L, Massmann H, Köster A, Hess B, Evers M, and von Figura K (1995). Mice deficient for the lysosomal proteinase cathepsin D exhibit progressive atrophy of the intestinal mucosa and profound destruction of lymphoid cells. *EMBO J* 14(15): 3599–3608.

44. Koike M, Shibata M, Ohsawa Y, Nakashii H, Koga T, Kametaka S, Waguri S, Momoi T, Kominami E, Peters C, Furaoka K von, Saftig P, and Uchiyama Y (2003). Involvement of two different cell death pathways in retinal atrophy of cathepsin D-deficient mice. *Mol Cell Neurosci* 22(2): 146–161.

45. Koike M, Nakashii H, Saftig P, Ezaki J, Ishikawa H, Ohsawa Y, Schubl-Schaeffer W, Watanabe T, Waguri S, Kametaka S, Shibata M, Yamamoto K, Kominami E, Peters C, von Figura K, and Uchiyama Y (2000). Cathepsin D deficiency induces lysosomal storage with ceroid lipofuscin in mouse CNS neurons. *J Neurosci Off J Soc Neurosci* 20(18): 6898–6906.

46. Koike M, Shibata M, Waguri S, Yoshimura K, Tanida I, Kominami E, Gotow T, Peters C, von Figura K, Mizushima N, Saftig P, and Uchiyama Y (2005). Participation of autophagy in storage of lysosomes in neurons from mouse models of neuronal ceroid-lipofuscinoses (Batten disease). *Am J Pathol* 167(6): 1713–1728.
47. Zhang D, Brankov M, Makhija MT, Robertson T, Helmerhorst E, Papadimitriou JM, and Rakocy PE (2005). Correlation between inactive cathepsin D expression and retinal changes in mcd2/mcd2 transgenic mice. Invest Ophthalmol Vis Sci 46(9): 3031–3038.

48. Steinfeld R, Reinhardt K, Schreiber K, Hillebrand M, Kraetzner R, Bruck W, Saftig P, and Gartner J (2006). Cathepsin D deficiency is associated with a human neurodegenerative disorder. Am J Hum Genet 78(6): 988–998.

49. Sirotola E, Partanen S, Strömpe P, Haapanen A, Haltia M, Maehlen J, Lobel P, and Johnson GS (2001). Congenital ovine neuronal ceroid lipofuscinosis—a cathepsin D deficiency with increased levels of the inactive enzyme. Eur J Paediatr Neurol EPN Off J Eur Paediatr Neurol Soc 5 Suppl A: 43–45.

50. Awano T, Kats ML, O’Brien DP, Taylor JF, Evans J, Khan S, Sohar I, Lobel P, and Johnson GS (2006). A mutation in the cathepsin D gene (CTSD) in American Bulldogs with neuronal ceroid lipofuscinosis. Mol Genet Metab 87(4): 341–348.

51. Awano T, Katz ML, O’Brien DP, Taylor JF, Evans J, Khan S, Sohar I, Lobel P, and Johnson GS (2006). A mutation in the cathepsin D gene (CTSD) in American Bulldogs with neuronal ceroid lipofuscinosis. Mol Genet Metab 87(4): 341–348.

52. Egberts F, Heinrich M, Jensen J-M, Winoto-Morbach S, Pfeiffer S, Wickel M, Schunk M, Steude J, Saftig P, Proksch E, and Schütze S (2004). Cathepsin D is involved in the regulation of translucinase 1 and epidermal differentiation. J Cell Sci 117(11): 2295–2307.

53. Chen SH, Arany I, Apisarnthanarax N, Rajaraman S, Tyring SK, Hori koshi T, Brysk H, and Brysk MM (2000). Response of keratinocytes from normal and psoriatic epidermis to interferon-gamma differs in the expression of zinc-alpha(2)-glycoprotein and cathepsin D. FASEB J Off Publ Fed Am Soc Exp Biol 14(3): 565–571.

54. Kawada A, Hara K, Kominami E, Hiruma M, Noguchi H, and Ishibashi A (2000). A cathepsin D-cleaved 16 kDa form of prolactin mediates postpartum cardiomyopathy. J Biol Chem 274(3): 430–435.

55. Zhou W, Scott SA, Shelton SB, and Crutcher KA (2004). Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. Cancer Lett 237(2): 167–179.

56. Fitzgerald PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, Ruby SG, O’Malley F, Simpson JF, Connolly JL, Hayes MD, Edge SB, Liu X, and Schnitt SJ (2000). Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 124(7): 966–978.

57. Bossard N, Descotes F, Bremond AG, Bobin Y, De Saint Hilaire P, Golffer F, Awada A, Mathevet PM, Berrard L, Barbier Y, and Estève J (2003). Keeping data continuous when analyzing the prognostic impact of a tumor marker: an example with cathepsin D in breast cancer. Breast Cancer Res Treat 82(1): 47–59.

58. Kirana C, Shi H, Laing E, Hood K, Miller R, Bethwaite P, Keating J, Jordan TW, Hayes M, and Stubbs R (2012). Cathepsin D Expression in Colorectal Cancer. From Proteomic Discovery through Validation Using Western Blotting, Immunohistochemistry, and Tissue Microarrays. Int J Proteomics 2012: 245819.

59. Shin SY, Sung NY, Lee YS, Kwon TS, Si Y, Lee YS, Oh ST, and Lee IK (2014). The expression of multiple proteins as prognostic factors in colorectal cancer: cathepsin D, p53, COX-2, epidermal growth factor receptor, C-erbB-2, and Ki-67. Gut Liver 8(1): 13–23.

60. Bossard N, Descotes F, Bremond AG, Bobin Y, De Saint Hilaire P, Golffer F, Awada A, Mathevet PM, Berrard L, Barbier Y, and Estève J (2003). Keeping data continuous when analyzing the prognostic impact of a tumor marker: an example with cathepsin D in breast cancer. Breast Cancer Res Treat 82(1): 47–59.

61. Sheikh AM, Li X, Wen G, Tauger Z, Brown WT, and Malik M (2010). Cathepsin D and apoptosis related proteins are elevated in the brain of autistic subjects. Neuroscience 165(2): 363–370.

62. Durán MC, Martín-Ventura JL, Mohammed S, Barderas MG, Blan coco-Colio LM, Mas S, Moral V, Ortega L, Tuñón J, Jensen ON, Vivanco F, and Egido J (2007). Atorvastatin modulates the profile of proteins released by human atherosclerotic plaques. Eur J Pharmacol 562(2): 119–129.

63. Grynd-Hansen M, Nylandsted J, and Jäättelä M (2004). Heat shock protein 70 promotes cancer cell viability by safeguarding lysosomal integrity. Cell Cycle 4: 1181–1186.

64. Palermo C and Joyce JA (2008). Cysteine cathepsin proteases as pharmacological targets in cancer. Trends Pharmacol Sci 29(1): 22–28.

65. LeTO G, Tuominen FM, Crescimanno M, Flandina C, and Gebbina N (2004). Cathepsin D expression levels in nongynecologic solid tumors: clinical and therapeutic implications. Clin Exp Metastasis 21(2): 91–106.

66. Liuadet-Coopman E, Beaujoquin M, Derooq C, Garcia M, Glondu Lassis M, Laurens-Matha V, Prébois C, Rochefort H, and Vignon F (2006). Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. Cancer Lett 237(2): 167–179.

67. Bossard N, Descotes F, Bremond AG, Bobin Y, De Saint Hilaire P, Golffer F, Awada A, Mathevet PM, Berrard L, Barbier Y, Estève J (2003). Keeping data continuous when analyzing the prognostic impact of a tumor marker: an example with cathepsin D in breast cancer. Breast Cancer Res Treat 82(1): 47–59.

68. Berchem G, Glondu M, Gleizes M, Brouillet J-P, Vignon F, Garcia M, Ruby SG, O’Malley F, Simpson JF, Connolly JL, Hayes MD, Edge SB, Liu X, and Schnitt SJ (2000). Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 124(7): 966–978.

69. Kirana C, Shi H, Laing E, Hood K, Miller R, Bethwaite P, Keating J, Jordan TW, Hayes M, and Stubbs R (2012). Cathepsin D Expression in Colorectal Cancer. From Proteomic Discovery through Validation Using Western Blotting, Immunohistochemistry, and Tissue Microarrays. Int J Proteomics 2012: 245819.

70. Shin SY, Sung NY, Lee YS, Kwon TS, Si Y, Lee YS, Oh ST, and Lee IK (2014). The expression of multiple proteins as prognostic factors in colorectal cancer: cathepsin D, p53, COX-2, epidermal growth factor receptor, C-erbB-2, and Ki-67. Gut Liver 8(1): 13–23.

71. Berchem G, Glondu M, Gleizes M, Brouillet J-P, Vignon F, Garcia M, Ruby SG, O’Malley F, Simpson JF, Connolly JL, Hayes MD, Edge SB, Liu X, and Schnitt SJ (2000). Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 124(7): 966–978.

72. Fusek M and Vetvicka V (1994). Mitogenic function of human procathepsin D: the role of the procathepsin D activation peptide in prostate cancer growth. The Prostate 24(1): 1–7.
77. Vetvicka V, Benes P, and Fusek M. (2002) Procathepsin D in breast cancer: what do we know? Effects of ribozymes and other inhibitors. Cancer Gene Ther 9(10): 854–863.

78. Vashishta A, Ohri SS, Proctor M, Fusek M, and Vetvicka V. (2006) Role of activation peptide of procathepsin D in proliferation and invasion of lung cancer cells. Anticancer Res 26(8): 4163–4170.

79. Hu L, Roth JM, Brooks P, Luty J, and Karpattin S. (2008) Thrombin up-regulates cathepsin D which enhances angiogenesis, growth, and metastasis. Cancer Res 68(12): 4666–4673.

80. Wolf M, Clark-Lewis I, Buri C, Langen H, Lis M, and Mazzucchelli L. (2003) Cathepsin D specifically cleaves the chemokines macrophage inflammatory protein-1 alpha, macrophage inflammatory protein-1 beta, and SLC that are expressed in human breast cancer. Am J Pathol 162(4): 1183–1190.

81. Vetvicka V, Vashistha A, Saraswat-Ohri S, and Vetvickova J. (2010) Procathepsin D and cancer: From molecular biology to clinical applications. World J Clin Oncol 1(1): 35–40.

82. Khalkhali-Ellis Z and Hendrix MJ. (2014) Two Faces of Cathepsin D: Physiological Guardian Angel and Pathological Demon. Biol Med 6(206): 2.

83. Briozzo P, Badet J, Capony F, Pieri I, Montcourrier P, Barritault D, Briozzo P, Badet J, Capony F, Pieri I, Montcourrier P, Barritault D, and Badet J. (2009) Cathepsin D activity and selectivity in the acidic conditions of a tumor microenvironment: Utilization in the development of a novel Cathepsin D substrate for simultaneous cancer diagnosis and therapy. Biochimie 95(11): 2010–2017.

84. Laurent-Matha S, Maruani-Herrmann S, Prébois C, Beaujouin M, Glondu M, Noël A, Alvarez-Gonzalez ML, Blacher S, Coopman P, Baghdiguian S, Gilles C, Loncarek J, Freiss G, Vignon F, and Liaudet-Coopman E. (2005) Cathepsin D levels in human cancer cells: an activator in the acidic conditions of human tumor microenvironment. J Cell Biol 168(3): 489–499.

85. Glondu M, Coopman P, Laurent-Matha S, Garcia M, Rochefort H, and Liaudet-Coopman E. (2001) A mutated cathepsin-D devoid of its catalytic activity stimulates the growth of cancer cells. Oncogene 20(47): 6920–6929.

86. Liaudet E, Derocq D, Rochefort H, and Garcia M. (1995) Transfect- ed cathepsin D stimulates high density cancer cell growth by inactivating secreted growth inhibitors. Cell Growth Differ Mol Biol J Am Assoc Cancer Res 6(9): 1045–1052.

87. Nirdé P, Derocq D, Maynadier M, Chambon M, Basile I, Gary-Bobo M, and Garcia M. (2010) Heat shock cognate 70 protein secretion as a new growth arrest signal for cancer cells. Oncogene 29(1): 117–127.

88. Febrerbaucher N, Tyurina Y, Poulsen B, Selhor U, Kallunki T, Bodes M, Weber E, Leist M, and Jäättelä M. (2004) Sensitization to the lysosomal cell death pathway upon immortalization and transformation. Cancer Res 64(15): 5301–5310.

89. Johansson A-C, Steen H, Ollinger K, and Roberg K. (2003) Cathepsin D mediates cytochrome c release and caspase activation in human fibroblast apoptosis induced by staurosporine. Cell Death Differ 10(11): 1253–1269.

90. Emter-Sedlak L, Shangary S, Rabinovitz A, Miranda MB, Delach SM, and Johnson DE. (2005) Involvement of cathepsin D in chemotherapeutic-induced cytochrome c release, caspase activation, and cell death. Mol Cancer Ther 4(5): 733–742.

91. Trincheri NF, Nicolta G, Follo C, Castino R, and Isidor C. (2007) Resveratrol induces cell death in colorectal cancer cells by a novel pathway involving lysosomal cathepsin D. Carcinogenesis 28(5): 922–931.

92. Bollmann R, Zheng L, and Stendahl O. (2007) Cathepsin-cleaved Bid promotes apoptosis in human neutrophils via oxidative stress-induced lysosomal membrane permeabilization. J Leukoc Biol 81(5): 1213–1223.

93. Bidère N, Lorenzo HK, Carmona S, Lafforge M, Harper F, Dumont C, and Senik A. (2003) Cathepsin D triggers Bax activation, resulting in selective apoptosis-inducing factor (AIF) relocation in T lymphocytes entering the early commitment phase to apoptosis. J Biol Chem 278(33): 31401–31411.

94. Beaujon M, Baghdiguian S, Glondu-Lassius M, Berchem G, and Liaudet-Coopman E. (2006) Overexpression of both catalytically active and -inactive cathepsin D by cancer cells enhances apoptosis-dependent chemo-sensitivity. Oncogene 25(13): 1967–1973.

95. Tardy C, Tynelâ J, Haslik A, Levade T, and Andrieu-Abadie N. (2003) Stress-induced apoptosis is impaired in cells with a lyosomal targeting defect but is not affected in cells synthesizing a catalytically inactive cathepsin D. Cell Death Differ 10(9): 1090–1100.

96. Schestokowa O, Geisel D, Jacob R, and Haslik A. (2007) The catalytically inactive precursor of cathepsin D induces apoptosis in human fibroblasts and HeLa cells. J Cell Biochem 101(6): 1558–1566.

97. Zhao S, Aviles ER, and Fujikawa DG. (2010) Nuclear translocation of mitochondrial cytochrome c, lysosomal cathepsins B and D, and three other death-promoting proteins within the first 60 minutes of generalized sequestration. J Neurosci Res 88(8): 1727–1737.

98. Baghdiguian S, Glondu L, Paffhausen T, Schwab M, and Westermann F. (2008) Cathepsin D promotes human neuroblastoma cells from doxorubicin-induced cell death. Carcinogenesis 29(10): 1869–1877.

99. Castino R, Bello N, Nicolta G, Follo C, Trincheri NF, and Isidoro C. (2007) Cathepsin D-Bax death pathway in oxidative stressed neuroblastoma cells. Free Radic Biol Med 42(9): 1305–1316.

100. Hah Y-S, Noh HS, Ha JH, Ahn JS, Hahm JR, Cho HY, and Kim DR. (2012) Cathepsin D inhibits oxidative stress-induced cell death via activation of autophagy in cancer cells. Cancer Lett 323(2): 208–214.

101. Marques C, Oliveira CSF, Alves S, Chaves SR, Coutinho OP, Corte-Real M, and Preto A. (2013) Acetate-induced apoptosis in colorectal carcinoma cells involves lysosomal membrane permeabilization and cathepsin D release. Cell Death Dis 4: e507.

102. Oliveira CSF, Pereira H, Alves S, Castro L, Baltazar F, Chaves SR, Preto A, Corte-Real M. (2015) Cathepsin D protects colorectal cancer cells from acetate-induced apoptosis through autophagy-independent degradation of damaged mitochondria. Cell Death Dis “in press”.

103. Hartwell LH. (2002) Nobel Lecture. Yeast and cancer. Biosci Rep 22(3-4): 373–394.

104. Diaz-Ruiz R, Uribe-Carvajal S, Devin A, and Rigoulet M. (2009) Cell death in yeast: growing applications of a dying buddy. Cell Death Differ 17(5): 733–742.
109. Hecht KA, Wyliaz VA, Ast T, Schuldiner M, and Brodsky JL (2013). Characterization of an M28 metalloprotease family member residing in the yeast vacuole. *FEMS Yeast Res* 13(5): 471–484.

110. Rupp S, Hirsch HH, and Wolf DH (1991). Biogenesis of the yeast vacuole (lysosome). Active site mutation in the vacuolar aspartic proteinase yscA blocks maturation of vacuolar proteinases. *FEBS Lett* 293(1-2): 62–66.

111. Hecht KA, O’Donnell AF, and Brodsky JL (2014). The proteolytic landscape of the yeast vacuole. *Cell Logist* 4(1): e28023.

112. Adams PDB, Mannarino SC, Riger CJ, Duarte G, Cruz A, Pereira MD, and Eleutherio ECA (2009). Lap4, a vacuolar aminopeptidase I, is involved in cadmium-glutathione metabolism. *Biomets Int J Role Met Ions Biochem Med* 22(2): 243–249.

113. Takeshige K, Baba M, Tsuboi S, Noda T, and Ohsumi Y (1999). Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol* 119(2): 301–311.

114. Kirisako T, Baba M, Ishihara N, Miyazawa K, Ohsumi M, Yoshimori T, Noda T, and Ohsumi Y (1999). Formation process of autophagosome is traced with Agp8p/Aut7p in yeast. *J Cell Biol* 147(3): 435–446.

115. Wolf DH and Elhmann C (1981). Carboxypeptidase S- and carboxypeptidase Y-deficient mutants of *Saccharomyces cerevisiae*. *J Bacteriol* 147(2): 418–426.

116. Van Den Hazel HB, Kielland-Brandt MC, and Winther JR (1996). Review: biosynthesis and function of yeast vacuolar proteinases. *Yeast* Chichester Engl 12(1): 1–16.

117. Mason DA, Shulga N, Undavai S, Ferrando-May E, Rexach MF, and Goldfarb DS (2005). Increased nuclear envelope permeability and Pep4p-dependent degradation of nucleoporins during hydrogen peroxide-induced cell death. *FEMS Yeast Res* 5(12): 1237–1251.

118. Marques M, Mojidea MA, Almeida T, Hohmann S, Moradas-Ferreira P, and Costa V (2006). The Pep4p vacuolar proteinase contributes to the turnover of oxidized proteins but Pep4p overexpression is not sufficient to increase chronological lifespan in *Saccharomyces cerevisiae*. *Microbiol Read Engl* 152(Pt 12): 3995–3605.

119. Gourlay CW and Ayscough KR (2006). Actin-induced hyperactivation of the Ras signaling pathway leads to apoptosis in *Saccharomyces cerevisiae*. *Mol Cell Biol* 26(17): 6487–6501.

120. Eastwood MD, Cheung SWT, Lee KY, Moffat J, and Meneghini MD (1981). Carboxypeptidase S- and carboxypeptidase Y-deficient mutants of *Saccharomyces cerevisiae*. *J Bacteriol* 147(3): 435–446.

121. Carmona-Gutiérrez D, Bauer MA, Ring J, Knauer H, Eisenberg T, and Madeo F (2010). Apoptosis in yeast: triggers, pathways, subroutines. *Cell Death Differ* 17(5): 763–773.

122. Pereira C, Silva RD, Saraiva L, Johansson B, Sousa MJ, and Corte-Real M (2008). Mitochondrion-dependent apoptosis in yeast. *Biochim Biophys Acta* 1783(7): 1286–1302.

123. Adamis PDB, Mannarino SC, Riger CJ, Duarte G, Cruz A, Pereira MD, and Eleutherio ECA (2009). The protective role of yeast cathepsin D in acetic acid-induced programmed cell death in *Saccharomyces cerevisiae*. *BMC Genomics* 14: 838.

124. Sousa M, Duarte AM, Fernandes TR, Chaves SR, Pacheco A, Leão C, Corte-Real M, and Sousa MJ (2013). Genome-wide identification of genes involved in the positive and negative regulation of acetic acid-induced programmed cell death in *Saccharomyces cerevisiae*. *BMC Genomics* 14: 838.