Compensational role between cathepsins

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Abstract

Cathepsins, a family of lysosomal peptidases, play a crucial role in maintaining cellular homeostasis by regulating protein turnover and degradation as well as many specific regulatory actions that are important for proper cell function and human health. Alterations in the activity and expression of cathepsins have been observed in many diseases such as cancer, inflammation, neurodegenerative disorders, bone remodelling-related conditions and others. These changes are not exclusively harmful, but rather appear to be a compensatory response on the lack of one cathepsin in order to maintain tissue integrity. The upregulation of specific cathepsins in response to the inhibition or dysfunction of other cathepsins suggests a fine-tuned system of proteolytic balance and understanding the compensatory role of cathepsins may improve therapeutic potential of cathepsin's inhibitors. Selectively targeting one cathepsin or modulating their activity could offer new treatment strategies for a number of diseases. This review emphasises the need for comprehensive research into cathepsin biology in the context of disease. The identification of the specific cathepsins involved in compensatory responses, the elucidation of the underlying molecular mechanisms and the development of targeted interventions could lead to innovative therapeutic approaches.

Keywords: lysosomal peptidases, compensation, cancer, bone resorption, inflammation, neurodegeneration

Abbreviations

AAA abdominal aortic aneurysm **AIA** antigen-induced arthritis **APCs** antigen-presenting cells **CIC-7** chloride channel 7 **CTLs** cytotoxic T lymphocytes **Ctsb**$^{-/-}$ cathepsin B deficiency **DCs** dendritic cells **EAE** experimental autoimmune encephalomyelitis **ECM** extracellular matrix **Ii** invariant chain **Mct** thyroid hormone membrane transporter **MDMs** peripheral blood monocytes **MEFs** mouse embryonic fibroblasts **MMP** matrix metalloprotease **NK** natural killer cell **NLS** nuclear localisation sequence **PrP**^{Sc} prion proteins **PyMT** polyoma middle T oncogene **T4** thyroxine **Tg** thyroglobulin prohormone **[VC(S)]** valine-citrulline linker

1. Introduction

Peptidases are one of the largest enzyme families in the human body and are involved numerous cell functions as well as in modulation of the cell environment [1]. In general they do not function independently, but are a part of complex proteolytic networks [2,3]. In these networks, they work either in linear pathways, regulatory loops or in cascades [2], in which peptidases regulate the activity of other peptidases, either directly or by cleaving other peptidase intermediates [4]. In particular, the interactions between different cathepsins can be observed already during their activation, as peptidases are synthesised as inactive zymogens and require cleavage of the propeptide prior to their function (discussed in [3]).

Cathepsins act as protein-degrading enzymes in lysosomes and also as important regulatory enzymes at other locations. In normal physiological processes, cathepsins' dependent proteolytic networks are tightly regulated at multiple levels to prevent pathologies caused by excessive and deregulated peptidase activities. Dysregulation of these networks, leading to altered expression and activity of cathepsins, is associated with a number of pathological processes such as cancer, neurodegeneration, immune disorders, osteoporosis and others. On this basis, cathepsins have been identified as biomarkers and as promising therapeutic targets for various diseases [5–16]. The expression and activity of cathepsins can be dysregulated by various mechanisms, including their increased expression, the presence of their endogenous inhibitors, subcellular localisation and changes in pH [17–19]. Number of studies demonstrated increased cathepsin activity in pathological processes which can be regulated at multiple levels e.g. with the use of small molecule inhibitors and neutralising antibodies. To date number of

cathepsin inhibitors have been identified and evaluated for their potential to impair cathepsin activity in various diseases (reviewed in [7,8,20–26]).

To maintain the constant proteolytic activity, redundancies and compensatory functions between different cathepsins have been observed. Understanding the compensatory mechanisms between the cathepsins is of great importance in order to apply them as therapeutic targets [3,17]. Furthermore, the compensation between different cathepsins makes the regulation by exogenous inhibitors even more complex. Successful targeting of the cathepsin of interest therefore requires a complete understanding of its function as well as its interaction within the proteolytic network and the possibilities of compensation of its activity by other cathepsins.

This review provides a comprehensive overview on the compensatory mechanisms between different cathepsins and emphasises the importance of understanding them for improved therapeutic interventions. Given the complexity of the peptidase network, compensation mechanisms may occur in different directions including multiple cathepsins. However, for the sake of clarity, the compensatory mechanisms between the cathepsins are presented in pairs.

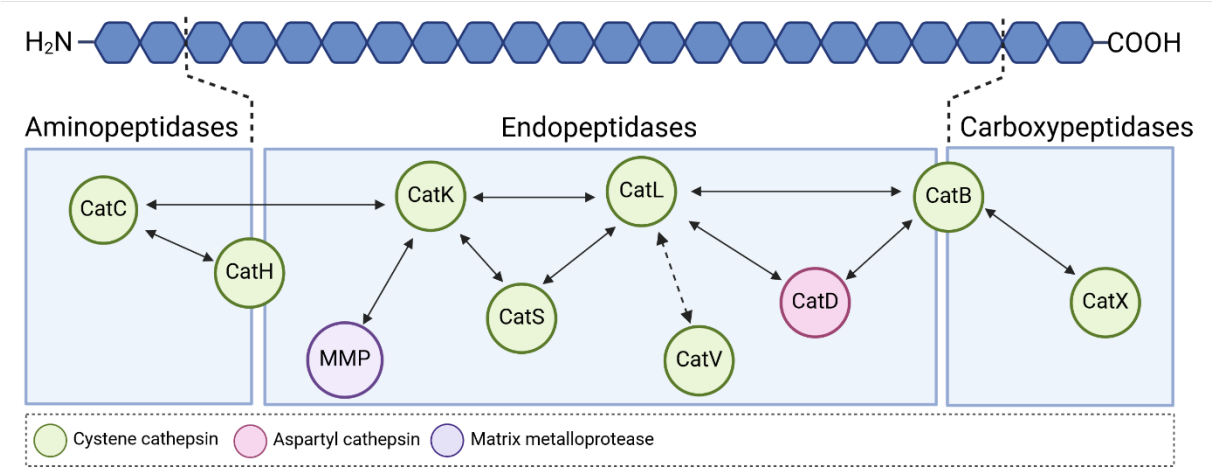

Figure 1. Schematic representation of compensatory pairs between different cathepsins. Cathepsin (Cat), matrix metalloprotease (MMP). Figure was created using BioRender.com.

2. Cathepsins B and X

The most obvious and by far the most extensively investigated compensation between two cathepsins is that between cathepsin B and X (Figure 1). Cathepsins B and X are unique among cysteine cathepsins in that they are the only two cathepsins with caboxypepetidase activity

[24,27–30]. Cathepsin B can also cleave substrates as an endopeptidase [24]. Its dual function can be attributed to the presence of an additional structural element known as the occluding loop [31]. The occluding loop is connected to the body of the enzyme at low pH within lysosomes by salt bridges and prevents access of the larger substrates to the active site of the enzyme. For this reason, cathepsin B acts as an exopeptidase (carboxydipeptidase) at low pH [31–36]. However, with increasing pH, a conformational change occurs in the occluding loop and the salt bridges are interrupted, allowing access of larger substrates to the active site of the enzyme and under these conditions cathepsin B acts as an endopeptidase [32–34]. Cathepsin X (also known as cathepsin Z, as it is encoded by Ctsz gene), on the other hand, has exclusively carboxymonopeptidase activity due to the presence of a three residue insertion motif that forms a characteristic "mini-loop" [28]. The "mini-loop" prevents access of longer substrates to the active site and determines its exclusive carboxypeptidase activity [28]. Compared to other cysteine cathepsins, cathepsin X also differs in the presence of a very short pro-region [28].

Cathepsin B is ubiquitously expressed and is involved in number of physiological processes from protein turnover to more specific regulatory processes. It is overexpressed in many pathological processes and is one of the most potent tumour-promoting cysteine peptidases (reviewed in [24,37]). On the other hand, the expression of cathepsin X in physiological processes is predominantly restricted to immune and neuronal cells. Nevertheless, increased expression of cathepsin X is also associated with a number of pathological processes in which it contributes via mechanisms other than extracellular matrix (ECM) degradation due to its exclusive carboxypeptidase activity (reviewed in [27]).

An increase in the expression and activity of cathepsin X following the loss of cathepsin B expression was first suggested by Vasiljeva et al. in a transgenic polyoma middle T oncogene (PyMT)-induced breast cancer mouse model (Table I). In this model with cathepsin B deficiency active cathepsin X was detected on the cell surface using the membrane-impermeable activity probe DCG-04 [38]. The compensatory effect between cathepsins B and X was confirmed in a transgenic breast cancer mouse model with cathepsin-B/-X-deficiency induced by the PyMT. The double deficiency of cathepsins B and X led to synergistic antitumour effects that significantly reduced tumour growth and metastasis. Single cathepsin B deficiency led to delayed detection of initial tumours and to a lower tumour burden, but only up to a certain stage. Cathepsin B deficiency was followed by an increased expression of cathepsin X, which obviously compensated for the loss of cathepsin B [38,39]. Furthermore, a separate study demonstrated that a double knockout of cathepsin B and X is needed to reduce

laser-induced choroidal neovascularisation, supporting the importance of the compensatory mechanism between related cathepsins also during angiogenesis [40]. The redundant function and compensatory role between the two cathepsins was also observed in epithelial–mesenchymal transition following induction of the reverse process, i.e. mesenchymal–epithelial transition, after simultaneous silencing of both cathepsins B and X in lung carcinoma cell line A549 [41]. Next, Akkari et al. observed that cathepsin X was also upregulated in response to the simultaneous deletion of cathepsins B and S [17]. In the RT2-PanNET mouse model, which lacks cathepsins B and S, the accumulation of macrophages with increased cathepsin X expression was observed in tumours. They showed that deletion of cathepsin X in $Cstb^{-/-}/Ctss^{-/-}$ mice reduced macrophage infiltration and tumour invasion [17]. In addition, increased cathepsin X activity and protein levels were detected after cathepsin B inhibition. These results suggest that the resistance that develops after cathepsin B-targeted antitumour therapy is due to the compensation by cathepsin X [42].

In addition to cancer, a compensatory role between cathepsins B and X has also been proposed during inflammation. It was observed in delayed-type hypersensitivity reaction, where topical treatment with the specific cathepsin B inhibitor CA-074 had a significant anti-inflammatory effect in acute but not in chronic delayed-type cutaneous hypersensitivity reaction. Despite the lack of cathepsin B, $Ctsb^{-/-}$ mice showed an enhanced inflammatory effect compared to wild-type mice during the acute delayed-type hypersensitivity reaction, which was assessed as increased ear swelling. Active site labelling and western blot analysis showed a compensatory increase in cathepsin X activity and protein levels in $Ctsb^{-/-}$ mice compared to wild type. Moreover cathepsin B was reciprocally increased in $Ctsz^{-/-}$ mice compared to the wild type [43].

3. Cathepsins B and D

Compensation of cathepsin B activity has also been demonstrated with cathepsin D (Figure 1, Table I). Cathepsin D is a lysosomal aspartyl endopeptidase that plays an important role in modulating the activity of various polypeptides, growth factors and enzymes [44]. In addition, cathepsin D plays an important role in the removal of aggregated and damaged proteins [44,45] and is one of the most important enzymes for maintaining the role of the autophagy-lysosome pathway [14,44,45]. Dysregulation of its expression and activity contributes to disease progression [14,44]. In neurodegeneration, cathepsin D was the first lysosomal peptidase reported to protect against α-synuclein aggregation and its cytotoxic effects. This was

confirmed in models with cathepsin D deficiency and those with cathepsin D overexpression, where cathepsin D deficiency promoted α-synuclein accumulation and its toxicity, while cathepsin D overexpression efficiently degraded α-synuclein [14,46–49].

In neurological disorders cathepsin B level and activity were increased in brains of $Ctsd^{-/-}$ mice [45,47]. Similarly, increased cathepsin B activity was observed by Carabtree et al. after lentiviral transduction with mutant cathepsin D, which led to a decrease in cathepsin D activity [50]. A dose-dependent increase in cathepsin B activity was also observed after inhibition of cathepsin D with pepstatin A [50]. However, the increase in cathepsin B activity cannot fully compensate for the loss of cathepsin D activity. Cathepsin D seems to be efficient in cleavage of α-synuclein [47,50], whereas an increase in cathepsin B is obviously not sufficient to maintain the degradation of α-synuclein, leading to its accumulation in brain [50]. The specific role of cathepsin D in the degradation of α-synuclein was also confirmed with the treatment with inhibitors, as pepstatin A, rather than a general inhibitor of cysteine peptidases E64 impairs α-synuclein degradation [50]. This shows that the activity of the aspartyl peptidase cathepsin D can only be partially compensated by the activity of cathepsin B.

Furthermore, the synergistic role of cathepsins B and D was also demonstrated in the autophagy of the pancreas as mice lacking both cathepsin B and D exhibited impaired autophagy, while it was not affected in mice lacking either cathepsin B or D. In $Ctsd^{-/-}$ mice, the expression of cathepsins B and L was also increased [51].

On the other hand, cathepsin D may regulate the activation of cathepsin B, which triggers processes that are downstream of proteolytic cascades [52]. For example, cathepsin B has been recognized as one of the most effective enzymes for trypsin activation [53–55]. Knockout of cathepsin D in mice reduced cathepsin B and further trypsinogen activation in pancreatic acinar cells [52]. The mechanism of cathepsin D maturation has also been shown to involve cathepsins B and L [56].

Taken together, these results point to the complex relationship between cathepsins B and D, which has been confirmed also in various other diseases such as neurodegeneration and cancer. especially in relation to the role of both cathepsins in the process of autophagy [14,44,45,51].

The lack of cathepsin D in mice may lead to an accumulation of mature cathepsins B and L [51,57]. Mehanna et al. also observed that the protein levels of cathepsin B and cathepsin L were increased in $Ctsd^{-/-}$ mice, but no significant change in mRNA levels or activity of either cathepsin was observed [57]. This suggests delayed processing of cathepsins B and L due to

cathepsin D deficiency [57]. Delayed activation of cathepsin B in cathepsin D knockout mice was also confirmed in the study by Aghdassi et al. [52]. On the other hand, the expression of cathepsin D was increased in mice lacking both cathepsin B and L [58].

Mehanna et al. also suggested that cathepsins D, B and L have the ability to degrade each other [57]. Furthermore, a cross-action between cathepsins has been shown in pathological processes in frontotemporal lobar degeneration (FTLD) and neuronal ceroid lipofuscinosis (NCL), a lysosomal storage disease associated with mutation in the progranulin gene and loss of progranulin function. Here it was shown that dysregulation of lysosomal homeostasis in microglia could trigger compensatory lysosomal changes in other brain cells, as the loss of progranulin leads to an accumulation of inactive cathepsins' pro-forms in microglia and increased expression, maturation and in vitro activity of cathepsins D, B and L, as shown in mouse embryonic fibroblasts and in brain extracts from aged mice with progranulin gene deficiency [59].

4. Cathepsins D and L

Redundancy with aspartyl cathepsin D is seen also for cysteine cathepsin L (Figure 1, Table I). This may explain the ability of cathepsin L to contribute to the clearance of pathological protein storage in neuronal ceroid lipofuscinosis (NCL), a childhood neurodegenerative disease characterised by impaired autophagic flux and pathological accumulation of proteins that is associated with cathepsin D deficiency. Here, in cells and *in vivo* in the mouse model deficient for cathepsin D, cathepsin L was able to cleave saponin C aggregates. Also, in mouse model deficient for cathepsin D a therapeutic clearance of protein aggregates after treatment with recombinant human procathepsin D was observed. In contrast, such an effect was not observed with cathepsin B, which acts as an exopeptidase in lysosomes under acidic conditions [60]. To further investigate relationship between cathepsins D and L and additionally cathepsin B Di Spiezio et al. performed an *in vitro* assay for cathepsin-mediated degradation of bovine serum albumin (BSA) [60]. The assay revealed effective degradation of BSA by cathepsins L and D, while cathepsin B was less efficient under lysosome-like conditions with a pH of 4.5. However, the most effective degradation of BSA was observed when all three enzymes were used in combination. The combined application of all three enzymes also led to an altered cleavage pattern. Taken together, although BSA is not a substrate that mimics specific disease model, this indicates a sequential, redundant and coordinated activity of the cathepsins and this findings could be useful for understanding interplay between cathepsins in pathological processes [60].

5. Cathepsins B and L

Besides cathepsin B, cathepsin L is the most ubiquitously expressed cysteine cathepsin. Cathepsin L acts solely as an endopeptidase [61]. Like cathepsin B, it ubiquitously present under physiological conditions in all organs. An increase in the expression and activity of cathepsins B and L, their translocation to the cell surface and their secretion are associated with a number of pathological processes in which they have many overlapping functions (see [5,14]). In line with this, compensational mechanism between cathepsins B and L can be observed at different levels. Compensation between cathepsins B and L (Figure 1) has been confirmed in mice lacking either cathepsin B or cathepsin L, with deficiency of one cathepsin resulting in only minor phenotypic defects (Table I) [58,62–65], while mice lacking both cathepsins die within two to four weeks after birth [58]. Felbor et al. showed that cathepsins B and L are required for the integrity of the postnatal central nervous system and that mice lacking both cathepsins exhibit histopathological changes in the central nervous system [58]. During this period, $Ctsb^{-/-}/Ctsl^{-/-}$ mice developed a pronounced lysosomal storage disease in the central nervous system and pronounced brain atrophy [58]. In mutant mice with double deficiency of cathepsin B and L, histopathological analysis revealed a stronger induction of autophagy based on the accumulation of lysosomal structures derived from autophagosomes, which contributes significantly to brain pathogenesis [66]. In addition, using subcellular proteomics Felbor group identify 19 proteins that are significantly increased in the lysosomes of the brains of mice with cathepsin B and cathepsin L double deficiency [67]. They include lysosomal enzymes as well as molecules associated with the ubiquitin-conjugating system, the prototypic secretory and endocytosed protein apolipoprotein E, neurochondrin, Rab14, carboxypeptidase E, calcyon, and the Delta/Notch-like epidermal growth factor-related receptor (DNER). Changes in the expression of these proteins indicate that cathepsins B and L are involved in biosynthesis and recycling processes during early postnatal brain development [67]. The compensatory function of cathepsins B and L was also confirmed by the drastic changes in secretome composition between wild-type mouse embryonic fibroblasts (MEFs) and $Ctsb^{-/-}/Ctsl^{-/-}$ MEFs. Cathepsin B and L double deficiency particularly affects matricellular proteins (dynamically expressed non-structural matrix proteins expressed in ECM) and proteins of the ECM and alters the expression of peptidases and their inhibitors [68]. Changes in the secretome composition were also observed in $Ctsl^{-/-}$ MEFs, affecting in particular the abundance of ECM composition, signalling proteins, other peptidases and endogenous peptidase inhibitors [69], while single cathepsin B deficiency had only limited effects on secretome composition [68]. Changes in proteome

composition were also observed in skin samples from Ctsb$^{-/-}$/Ctsl$^{-/-}$ mice [70]. Taken together, all these results indicate a strong compensatory effect between cathepsin B and L [68]. In MEFs lacking cathepsins B and L, an accumulation of enlarged Lamp-1-positive vesicles was observed [68]. A minor lysosomal accumulation of acidic Lamp-1-positive vesicles was observed also in MEFs with a single deficiency of cathepsin B or L, despite mice showed no difference in phenotype [68]. Biological processes that are impaired by a double deficiency of cathepsins B and L include cell adhesion, migration, ECM remodelling, signalling and proteolytic processing, which are particularly important during cancer progression [69].

Cathepsin B and L can both degrade abnormal isoforms of prion proteins (PrP^{Sc}) in neuronal cells during scrapie infection. Here, a compensation between both cathepsins was observed, as after simultaneous inhibition of both cathepsins by siRNA, the degradation of abnormal isoforms of PrP^{Sc} was increased in the scrapie-infected cells, while inhibition of a single cathepsin by siRNA still resulted in a decreased amount of PrP^{Sc} in the cells. This is the result of the presence of an intrinsic compensatory mechanism in the cells, due to which a decrease in level of one cathepsin could cause a compensatory increase in the activity of the other cathepsin. A similar effect was not observed when individual cathepsin was blocked by specific inhibitors. In contrast to inhibition of the expression of individual cathepsins, inhibition of cathepsin B or L activity by inhibitors led to a marked increase in PrP^{Sc} in scrapie-infected GT1-1 cells [71].

An overlapping function of cathepsins B and L has also been demonstrated in the processing of β-protryptase in human mast cells. Both, silencing or inhibition of cathepsin B or L impair the processing of protryptase. However, it is not clear why no compensation between the two cathepsins was observed during this process [72].

In pancreatitis, cathepsin B activates trypsinogen and supports the intracellular peptidase activation cascade. On the other hand, cathepsin L has been identified as a potent inactivator of trypsinogen and trypsin. Thus, cathepsin L may act as cathepsin B antagonist in the digestive peptidase cascade that triggers pancreatitis. However, cathepsin L influences the severity of the disease through its anti-apoptotic function [73]. A similar observation was obtained for trypsinogen activation in macrophages, where cathepsins B and L also have opposing functions, as trypsin activity was significantly reduced in macrophages from Ctsb$^{-/-}$ mice, while it was increased in macrophages from Ctsl$^{-/-}$ mice [74]. In addition, Chen at al. showed that double deletion of cathepsin B and L in the pancreas altered intrapancreatic trypsin activity but had no effect on disease severity and inflammatory response after induction of acute pancreatitis [75].

During cancer progression, higher levels of processed cathepsin L were also observed in Ctsb$^{-/-}$ tumours in the mouse model for pancreatic cancer. Loss of cathepsin B leads to decreased mitogen-activated protein kinase (MAPK) signalling in pancreatic cells, with the decrease in phospho-Erk correlating with an increase in levels of active cathepsin L [76]. Cathepsin L was found to compensate for the loss of cathepsin B activity in tumours [77]. Furthermore, the use of antibody-drug conjugates in which the valine-citrulline [VC(S)] linker was designed to be specifically cleaved by cathepsin B during transport to lysosomes revealed that other cysteine cathepsins, including cathepsin L, can cleave the VC(S) linker and are able to compensate for the loss of cathepsin B activity, as demonstrated by mass spectroscopy analysis of the cleavage products of antibody-drug conjugates [77].

Next, Mizunoe at al. showed that cathepsin L activity was compensated by increasing cathepsin B activity also in adipocytes. In this case the downregulation of cathepsin L suppressed adipocyte differentiation while compensatory activation of cathepsin B induced autophagosome accumulation [78]. At the same time, upregulation of cathepsin B inhibited cathepsin L activity, and after treatment with the selective cathepsin B inhibitor CA-074Me, cathepsin L activity was restored. Suppression of cathepsin L by a selective inhibitor (Z-FY-CHO) in 3T3L1 adipocytes and obese white adipose tissue also led to a compensatory increase in cathepsin B activity and transcriptional upregulation. It can therefore be concluded that dysregulation of cathepsin L leads to a compensatory upregulation of cathepsin B [78].

The compensatory role of cathepsins B and L has recently been identified in SARS-CoV-2 virus entry into host cells, with both cathepsins involved in receptor-mediated endocytosis of spike protein activation [79–83]. Cathepsin L is a host cysteine peptidase most commonly associated with the activation of viral glycoproteins and has the ability to process the SARS-CoV-2 spike protein and related proteins of other human CoVs [79,84–88]. However, involvement in receptor-mediated endocytosis of SARS-CoV-2 by cleavage of spike protein has also been demonstrated for cathepsin B [79,89–91]. For selective cathepsin B and L inhibitors, it has been shown that their antiviral activity depends on the cell type and correlates well with the intracellular amount and activity of the targeted cathepsin. The contribution of cathepsin B and L to viral glycoprotein activation thus depends on their intracellular quantity and activity [79]. Moreover, synergistic activity with the serine peptidase TMPRSS2 in mediating viral entry has been observed in SARS-CoV-2 infection, creating two independent entry pathways for the SARS-CoV-2 virus into host cells [81–83,92].

Furthermore, Ebert et al. identified cathepsins B and L as endocytic peptidases required for reovirus entry into murine fibroblasts [93]. Inhibition of cathepsin L activity by the specific inhibitor Z-Phe-Tyr(t-Bu)-diazomethylketone or its removal resulted in inefficient proteolytic degradation of the viral capsid proteins, whereas inhibition of cathepsin B activity by the specific inhibitor CA-074 or its absence still supported reovirus disassembly and growth. This suggests that cathepsin L is more effective in mediating reovirus degradation compared to cathepsin B. However, complete suppression of the activity of both cathepsins B and L resulted in a complete abrogation of reovirus disassembly and growth, confirming the compensatory role between the cathepsins [93].

Taken together this shows that cathepsins B and L can compensate for each other's activity at different levels in physiological and pathological processes as a part of proteolytic network.

6. Cathepsin S and L

Cathepsin S is another lysosomal cysteine endopeptidase. In contrast to most cathepsins, the activity of cathepsin S is maintained under physiological conditions at neutral and moderately alkaline pH [94,95]. In addition, the proform of cathepsin S is autocatalytically processed at neutral pH in the presence of glycosaminoglycans [96]. Cathepsin S is not ubiquitously expressed, its expression is restricted to cells and tissues such as the spleen and lymphatic system, where it is mainly expressed in immune cells such as macrophages and professional antigen-presenting cells (APCs) including B cells and dendritic cells (DCs), indicating the important role of cathepsin S in the immune response, where it contributes to antigen processing and presentation and is involved in the differentiation of immune cells [14,94,95]. Furthermore, cathepsin S is important for mediating autophagy and is involved in the process of apoptosis. Similar to other cathepsins with endopeptidase activity, cathepsin S contributes to ECM degradation and is involved in cell signalling. Cathepsin S has also been shown to promote angiogenesis and microvessel formation in physiological neovascularisation through the degradation of anti-angiogenic peptides and the release of proangiogenic factors [97].

Non-redundant roles in the immune system have been proposed for cathepsin S. It is required for the efficient presentation of a variety of antigens in DCs and B cells, which do not normally express cathepsin L [98]. However, cathepsin S has been suggested to regulate protein levels of mature cathepsin L in B in cells, with protein levels shown to increase in the absence of

cathepsin S. It is suggested that this is due to the degradation of mature cathepsin L by cathepsin S. This degradation can vary between different APCs [99].

The redundancy and compensatory activity of cathepsins S and L is mainly related to their function in antigen presentation (Figure 1, Table I). Both cathepsin S and cathepsin L are involved in the differentiation of the Th17 subset of helper lymphocytes, which play an important role in cancer-related inflammation. They have also been proposed as key enzymes involved in invariant chain (Ii) processing and assembly of MHC class II molecules [7,62,100–105]. The absence of cathepsins S and L impairs the onset of the humoral immune response and the selection of T cells [105]. Moreover, cathepsins S and L are regulated by the endogenous cysteine cathepsin inhibitor cystatin F, which is an important immunosuppressive factor in natural killer (NK) cells and cytotoxic T lymphocytes (CTLs). Cystatin F inhibits cathepsin S in immature dendritic cells and cathepsin L activity in maturing adherent dendritic cells [105].

During antigen processing, expression of both cathepsin L and S in embryonic fibroblast lines mediates Ii chain processing, but different subsets of peptides are generated or destroyed in the presence of cathepsin L or S [103]. In macrophages expressing both cathepsin L and S, different regulation of the two enzymes was observed in the presence of interferon-γ. In the presence of this cytokine, the activity of cathepsin L is reduced, despite its high protein levels, indicating its specific inhibition. On the other hand, the activity of cathepsin S increases, and cathepsin S is preferentially used for the presentation of MHC class II in APC [104].

In addition, Gresser et al. identified cathepsins L and S as key components in the regulation of the immune potential of astrocytes and microglia [106]. In these cells, they are involved in the degradation of the Ii chain. In immunocompetent astrocytes and microglia, the expression of cathepsins L and S was impaired, which led to changes in the processing of the invariant chain [106].

The redundancy between cathepsins S and L and additionally cathepsin B has also been demonstrated in experimental autoimmune encephalomyelitis (EAE), the most commonly used animal model to study the immune processes associated with multiple sclerosis [98]. $Ctsb^{-/-}$ /$Ctss^{-/-}$ mice were completely susceptible to EAE, while $Ctsl^{-/-}$ mice were protected from EAE. Cathepsins S and L are co-expressed by macrophages and have similar cleavage sites, so it is possible that the activity of cathepsin L compensates for the absence of cathepsin S. Furthermore, in an experimental model of EAE, simultaneous deficiency of cathepsin B and S leads to protection against EAE with significantly reduced MHC-II expression and MOG

antigen presentation efficiency, also indicating a compensatory function between cathepsin B and cathepsin S activity [98]. Studies on experimental EAE models suggest that both cathepsin B and cathepsin L are necessary but not sufficient to compensate for the loss of cathepsin S in macrophages. This suggests that inhibition of multiple cathepsins is required to modulate autoimmune diseases [98].

A compensatory effect leading to increased levels of active cathepsin S with simultaneous inhibition of cathepsin L was observed following treatment of breast cancer cells with the broad-spectrum cathepsin inhibitors E-64 and protein inhibitor cystatin C [107]. The activity of cathepsin L was inhibited by E-64 and cystatin C, while the inhibitors did not affect the activity of cathepsin S and surprisingly increased levels of active cathepsin S were observed after treatment with inhibitors. The different response to treatment of breast cancer cells with inhibitors could be a consequence of differences in cathepsin L and S localisation, as cathepsin L is localised in the cytoplasm and extracellularly, while cathepsin S is predominantly localised in vesicles (lysosomes, endosomes) [107].

In addition, cathepsins L and S were proposed to be involved in the processing and activation of pro-cathepsin C (pro-dipeptidyl peptidase I) to its active form [108]. However, it was later demonstrated using $Ctsl^{-/-}/Ctss^{-/-}$ mice that neither cathepsin L nor S is required for cathepsin C activation and that one or more additional peptidases are involved in this process [109].

Veillard et al. observed the involvement of cathepsin S and L in the regulation of the same process in modulating the anti-angiogenic activities of human endostatin [110]. They demonstrated that cathepsins S and L release two peptides from endostatin that have enhanced angiogenic properties, while silencing of cathepsins S and L impaired the degradation of endostatin [110]. On the other hand, cathepsin L was also reported to be involved in generation of endostatin by proteolytic cleavage of collagen XVIII as was first demonstrated on the murine hemangioendothelioma (EOMA) cells model [111]. In addition, cathepsins B, L and S were found to regulate the initiation of pancreatitis through premature trypsinogen activation in acniar cells [112].

7. Cathepsins K and L

Cathepsin K is a cysteine cathepsin that is highly expressed in osteoclasts and secreted into the resorption lacunae [113] where an acidic pH prevails, which is essential for the demineralisation process of the bone matrix and for the activity of cathepsin K [114]. Cathepsin K degrades type

I collagen, osteopontin and osteonectin, the proteins of the bone matrix [114,115]. The collagenous matrix is digested in resorption lacunae, its smaller fragments are also phagocytosed and then digested intracellularly [116]. The importance of cathepsin K for bone remodelling has been demonstrated in three ways: 1) a cathepsin K-specific inhibitor inhibited osteoclast resorption *in vitro* and *in vivo* [117], 2) a mutation in the cathepsin K gene is responsible for abnormal bone remodelling in patients with pycnodysostosis; in these patients there are substantial areas of undigested bone matrix [116] and 3) osteoclasts from $Ctsk^{-/-}$ mice can demineralise bone matrix but are unable to degrade it [118]. Since bone slices from these mice showed no cathepsin activity, Dodds et al. concluded that cathepsin K is a predominant cysteine peptidase of the osteoclast [114]. They also showed that procathepsin K is converted to the mature form as the osteoclast approaches the bone and is then released into the resorbing compartment at the ruffled osteoclast border.

Homozygous cathepsin K knockout mice have been produced by several groups (e. g. [118–120]) to investigate the role of cathepsin K in bone resorption. The abnormalities in these mice affect most bones with rapid remodelling such as long bones and vertebrae [118] and are similar to the abnormalities in patients with pycnodysostosis [119]. When comparing the osteopetrotic phenotype between $Ctsk^{-/-}$ mice and patients with pycnodysostosis, the latter were found to have a more severe phenotype [120]. In addition, the osteopetrotic phenotype is less severe in $Ctsk^{-/-}$ mice compared to other knockout mice with disruptions in osteoclast differentiation or function unrelated to cathepsin K [120]. Kiviranta et al. have described several compensatory mechanisms in $Ctsk^{-/-}$ mice that mitigate the absence of cathepsin K. Besides the increase in the number of osteoclasts and the increase in osteoclast surface area, the upregulation of mRNA levels for other peptidases is an important compensatory mechanism (both in homozygous and heterozygous cathepsin K-deficient mice) [120]. In long bones they detected increased mRNA levels for cathepsin L, MMP-9 (matrix metalloprotease 9), MMP-13, MMP-14 (MT1-MMP) and TRACP (tartrate-resistant acid phosphatase) (Figure 1, Table I). The difference in mRNA levels was more pronounced in homozygous cathepsin K-deficient mice than in heterozygous mice, in which only MMP-13 mRNA was significantly higher.

The mRNA concentration of cathepsin L is increased by approx. 30% in homozygous cathepsin K-deficient mice [120]. Cathepsin L is a lysosomal endopeptidase, that is ubiquitously distributed and activated at acidic pH (in lysosomes) [121]. In bone, it is found in osteoclasts where its intracellular level is extremely variable and much lower than the level of cathepsin K [122,123]. Cathepsin L was also found to be secreted by osteoclasts as well as by chondrocytes,

macrophages, synovial fibroblasts and tumour cells. Cathepsin L degrades several bone matrix proteins such as type I, II, IV, IX and XI collagens and gelatine. It cannot cleave the triple helical domain of collagen I like cathepsin K, but cleaves the non-helical telopeptide region of triple helical type I and II collagens as well as cartilage-residing proteoglycans such as aggrecan (summarized from [124]). The expression of cathepsin L is upregulated under pathological conditions, many of which are associated with altered bone remodelling (e.g. osteoporosis, rheumatoid arthritis, osteoarthritis). Under these conditions it can therefore support bone remodelling in cooperation with cathepsin K, MMPs and serine peptidases [124].

Several groups have shown that the intracellular activity and secretion of cathepsin L increases *in vitro* under the influence of parathyroid hormone, c-Src tyrosine kinase and proinflammatory cytokines (interleukin 1α, 1β and 6, TNFα and IFNγ). In addition, they also stimulate the secretion of procathepsin L in the culture medium from a bone cell mixture, where it is rapidly activated under acidic conditions (summarized from [124]). Cathepsin L is also responsible for the activation of other proteolytic enzymes such as MMPs, urokinase-type plasminogen activator (uPA) and heparinase, which are known to promote bone remodelling processes (summarized from [124]).

Jansen et al. investigated cathepsin K deficiency in lactating women, who have a higher need for calcium that originates in bone. Higher bone resorption is achieved by upregulation of osteoclastogenesis and osteolysis mediated by osteocytes, in which the expression of cathepsin K is also increased in lactating mice [125,126]. Despite the cathepsin K deficiency in a lactating woman with pycnodysostosis, the researchers found a physiological increase in bone resorption, while serum levels of bone resorption marker CTX were similar to healthy women and the number of osteoclasts remained the same. The patient had a low expression of cathepsin K mRNA, but during lactation the expression of cathepsin L and cathepsin S was strongly upregulated in the osteoclasts. After weaning, however, they dropped to the control values of healthy women [126].

Several studies have provided mixed results on the role of cathepsin L in bone resorption. One study found a reduction in trabecular bone in $Ctsl^{-/-}$ mice [127], while another study using cathepsin L inhibitors showed that cathepsin L is not involved in osteoclastic bone resorption of the long bone system [128], and another study found neither an osteoclastic effect on bone nor evidence of an osteopetrotic phenotype in $Ctsl^{-/-}$ or $Ctsb^{-/-}$ mice [123]. When MMPs inhibitors were used in $Ctsb^{-/-}$ calvarial bones, there was no effect on osteoclastic resorption, as

if MMPs are not utilised by the osteoclasts under these conditions implying that cathepsin L is necessary for the MMP activation [123].

Thyroglobulin prohormone (Tg) is synthesised in thyroid epithelial cells, transported to their apical plasma membrane and exocytosed into the follicular lumen, where it is covalently cross-linked and stored. When required, the covalently cross-linked Tg is first brought into solution and then partially degraded to release the hormone thyroxine (T4) (summarized from [129]). It was found that several cathepsins are important for these processes and thus enable the thyroid gland to function properly. Using mice with cathepsin deficiency and double deficiency Friedrichs et al. showed that cathepsin B and cathepsin L play a role in the solubilization of Tg while cathepsin K and cathepsin L are necessary for its partial proteolysis and the release of T4 [129]. Mice lacking cathepsin K or cathepsin K/cathepsin B have normal T4 levels because cathepsin L is overexpressed in thyroid epithelial cells. Mice lacking cathepsin L or cathepsin L/cathepsin K have very low serum T4 levels. Since T4 was still present, they proposed several other cathepsin candidates for proteolytic degradation of Tg. They found elevated levels of an aspartic peptidase cathepsin D, albeit in an inactive proform, which seems to be an unlikely candidate, as well as cathepsin S. In a later study another cathepsin, cathepsin H, was found to be expressed by rat FRTL-5 thyroid cells after their induction with follicular Tg mRNA. Subsequently, cathepsin H is concentrated together with internalised Tg in lysosomes. The group showed that a certain concentration of follicular Tg activates cathepsin H-mediated degradation of Tg in lysosomes [130].

Venugopalan et al. investigated mechanism of compensation of cathepsin K by cathepsin L in the thyroid gland in mice with single, double and triple deficiencies of cathepsin K and the thyroid hormone membrane transporters Mct8 and Mct10, which are important for the release of thyroid hormone into the blood and its subsequent uptake into peripheral organs [131]. Cathepsin L was only slightly overexpressed in thyroid tissue lysates from $Ctsk^{-/-}$ mice but strongly overexpressed in double and triple knock-outs ($Ctsk^{-/-}$/$Mct8^{-/-}$ and $Ctsk^{-/-}$/$Mct8^{-/-}$/$Mct10^{-/-}$). They discovered a compensatory regulation between cathepsin K and cathepsin L and a crosstalk between cathepsins and both transporters [131,132]. The maturation and degradation processes of cathepsins were unchanged, but there was a higher number of lysosomes per cell, indicating increased lysosome biogenesis. Indeed, they showed that in $Mct8^{-/-}$ and $Mct10^{-/-}$ mice, intracellular thyroid hormone levels increase and this hyperthyroid state increases ROS levels, which promotes autophagy and lysosomal biogenesis. Elevated TH itself also induces lysosomal activity and autophagy and activates the transcription factor EB (TFEB),

which in turn increases the transcription of cathepsin genes and induces lysosomal biogenesis [131].

In inflammatory diseases, macrophages accumulate and secrete cathepsins K, L and S, which destroy elastin-rich tissues [133]. *In vitro* experiments with macrophages differentiated from peripheral blood monocytes (MDMs) under conditions similar to the sites of tissue damage *in vivo* showed an upregulation of cathepsin expression and secretion. While cathepsins K, B, S and D were initially secreted as proforms and later after a few days in active form, cathepsin L was secreted in active form from the beginning [133]. At the same time, the expression of components of the vacuolar-type H^+-ATPase also increased and the enzyme was mobilised to the macrophage plasma membrane to create a pericellular acidic environment for cathepsin activity. The same group also prepared $Ctsk^{-/-}$ MDMs and found that their elastinolytic ability was comparable to that of control macrophages and concluded that cathepsin L and/or cathepsin S compensated for the loss of cathepsin K [133].

Abdominal aortic aneurysm (AAA) is a state of chronic inflammation with upregulated expression of several proteolytic enzymes (cathepsins B, D, K, L and S) in various cells of the arterial wall with the highest expression in macrophages [134]. Cathepsins are responsible for excessive degradation of the extracellular matrix, which is a primary mechanism in the development of atherosclerotic cardiovascular diseases. They are also involved in the cellular uptake and metabolism of lipids and in the modulation of cholesterol efflux [135]. Cathepsin K is an important enzyme in AAA formation due to its strong elastolytic and collagenolytic activities and its upregulated expression in aneurysm lesions [136,137]. The role of cathepsin K in aneurysms was investigated in apolipoprotein E-deficient mouse models of AAA $apoE^{-/-}$ and $Ctsk^{-/-}/apoE^{-/-}$ in which aneurysm formation was induced by sustained subcutaneous infusion of angiotensin II [138]. Bai et al. found that the absence of cathepsin K did not reduce aneurysm development, size or severity, nor did it reduce elastic lamina degradation due to upregulation of cathepsins S and C by macrophages [138]. They also showed that after angiotensin II infusion the number of granulocytes and effector T-helper cells increased in $Ctsk^{-/-}/ApoE^{-/-}$ mice [138]. In another study experiments were performed in wild-type and $Ctsk^{-/-}$ mice with experimental AAA induced by aortic perfusion with pancreatic elastase [139]. They showed that cathepsin K is important for the proliferation of CD4+ T-helper cells in aortic lesions and that in the absence of cathepsin K, the expression of cathepsin L is increased in these lesions, but not its activity; at the same time, the expression of MMP-2 is decreased. Nevertheless, the activity of cathepsin L is increased in endothelial cells of $Ctsk^{-/-}$ mice and

compensates for its absence [139]. The same group investigated the role of cathepsin L in a $Ctsl^{-/-}$ mouse model of AAA (induced by elastase perfusion) and showed that the absence of cathepsin L decreases the expression of cathepsin S, cathepsin K and MMPs 2 and 9 in different cell types and apparently regulates their expression and activity [140]. They also pointed out several differences between the two cathepsins: cathepsin L does not affect the proliferation of T cells like cathepsin K, but promotes their migration and the migration of monocytes through collagen and through an endothelial cell monolayer and collagen in a transwell experiment. Cathepsin K is also important for apoptosis of smooth muscle cells whereas cathepsin L is not [140].

8. Cathepsin K and MMPs

Matrix metalloproteinases (MMPs) were initially recognised as a group of collagenolytic enzymes responsible for the degradation of extracellular matrix elements, but were later shown to have many functions for tissues, including osteoclast function, where they degrade collagen extracellularly or are involved in receptor-mediated collagen uptake, among others (the role of MMPs in bone is nicely reviewed by [141] and [142]). They are active at neutral pH. Using inhibitors for MMPs and cysteine peptidases, it has been suggested that both cysteine peptidases and MMPs are necessary for the degradation of demineralised collagen, but that they act sequentially in different steps and that their action depends on bone type [141]. MMPs are important for the initial and final steps, while cathepsins are important for the main dissolution of the organic bone matrix [120,143]. One of hypotheses for the compensation of the loss of cathepsin K by MMPs is supported by the fact that the type I collagen fragments produced by MMPs (C-terminal ICTP) are increased in the serum of patients with pycnodysostosis (Table I), while the fragments produced by cathepsin K (C-terminal CTX) are reduced [144]. In addition, mononuclear ICAM-positive cells (the so-called bone lining cells) begin resorption of non-mineralised collagen protruding from the bone surface before the osteoclasts arrive at the site and then complete the degradation of the collagen leftovers [143,145]. Everts et al. have shown by electron microscopy that collagen fibrils are enwrapped by these bone lining cells and that this type of collagen removal is a characteristic of MMPs [143]. However, cysteine peptidases and MMPs are involved in the process of bone resorption by the osteoclasts [143]. In the absence of cathepsin K, bone lining cells enter areas of undigested bone and increase their activity compensating for the loss of cathepsin K. In contrast, during remodelling of adult connective tissue by fibroblasts, MMP-14 binds to degraded collagen fibrils and induces

collagen phagocytosis. The collagen is then further degraded in the lysosome by cathepsin B [146]. Several MMPs are collagenolytic, including MMPs 2, 9, 13 and 14 which act in bone, but only MMP-13 plays a role in bone resorption, as shown in experiments with knockout mice (for a review see [141]). Other MMPs are required for osteoclast migration. *In vitro* experiments have shown that osteoclastic MMPs are essential for osteoclast migration, as osteoclasts can migrate through collagen in the absence of other cells [147]. MMP-9 and MMP-14, for example, have been shown to be important for the recruitment of preosteoclasts from the mesenchyme into long bones, with MMP-9 co-operating with MMP-13 [148,149]. MMP-9 is able to weakly degrade type 2 collagen from demineralised cartilage, with efficiency being doubled when it cooperates with MMP-13 [149], and furthermore, proteolytic activity in MMP-$9^{-/-}$ mice can be compensated by higher expression of MMP-13 in osteoclasts and chondrocytes [150]. Bone turnover slows down during ageing and older mice cope better with cathepsin K deficit [120].

Everts et al. demonstrated that there are different osteoclasts in two different parts of the skeleton, the long bones and the skull bones. The osteoclasts of the long bones express much more cathepsin K which appears to be much more important for the resorption of the long bones, while MMPs and other cysteine peptidases appear to play a greater role in the skull bones. Selective MMPs inhibitors inhibited bone matrix resorption in cranial bone explants, whereas this was not the case in long bones [123]. However, when cathepsin K expression was suppressed, they showed that MMPs are also involved in the resorption of long bones.

The involvement of cathepsin K and MMPs was also investigated in calcified and decalcified slices of cortical bovine bone emphasising the importance of pH [151]. While the pH range of cathepsin K activity is between acidic (4.0) and neutral [115,152], that of MMPs is neutral [153]. They showed a vesicular localisation of cathepsin K and chloride channel 7 (CIC-7) in osteoclasts (produced from monocytes from the peripheral blood of healthy donors) seeded on decalcified bone, whereas on calcified bone they were localised in gradients towards the resorption lacuna. In contrast, MMPs 9 and 14 localised in gradients towards the matrix and on the cell surface in both calcified and decalcified bone. This could indicate a lesser importance of the acidification process in decalcified bone and a lesser role of cathepsin K. Next, they used osteoclasts produced from monocytes of ADOII (autosomal dominant osteopetrosis type II) patients' and showed that they are less able to degrade the decalcified matrix. Using different inhibitors (for cathepsin K, MMPs, CIC-7 and V-ATPase), they confirmed acidification dependent cathepsin K-mediated degradation of the organic matrix and showed that MMPs not

only degrade the collagen of calvariae, but also the collagen of long bones when required, but only when cathepsin K activity is weakened. MMPs are therefore able to compensate for the loss of cathepsin K. These MMPs, however, are mainly expressed by osteoblasts, but also by osteoclasts [151].

The process of bone resorption is also important for tooth eruption and morphogenesis [154], as the alveolar bone surfaces of wild-type mice are rich in active osteoclasts. Radiological and histological analyses of the craniofacial complex and tooth eruption in $Ctsk^{-/-}$ mice, however, showed normal morphogenesis, whereas the long bones of these mice suffered from impaired bone resorption and resembled those typical of pycnodysostosis patients [155]. They proposed two compensatory processes: the increased number of osteoclasts in $Ctsk^{-/-}$ mice and the increased level of MMP-9 in osteoclasts compared to wild-type mice. Later studies found increased expression of MMP-2 [156] and MMP-1 [157] which are involved in the remodelling of extracellular matrix during different phases of tooth eruption. However, they did not investigate potential compensatory mechanisms.

9. Cathepsins V and L

Cathepsin V (also known as cathepsin L2) is a cathepsin L-like peptidase that is the result of a gene duplication. It has about 75% homology with cathepsin L, but has a much more restricted expression pattern than cathepsin L [158]. It has no murine counterpart. Both murine and human cathepsin L are ubiquitously expressed, whereas cathepsin V expression is restricted to macrophages, cortical thymic epithelial cells, testis, corneal epithelium and epidermis. However, only murine cathepsin L and human cathepsin V are elastases [159,160]. For these enzymes, no compensation in the sense mentioned has been described so far, but several studies have shown that their activities can at least partially substitute each other (Figure 1, Table I)(reviewed in [161]). To investigate the role of cathepsin V, Hageman et al. prepared $Ctsl^{-/-}$ mice and $Ctsl^{-/-}$ mice with human cathepsin V expressed under the control of the K14 promoter, which directs expression to some epithelial cell types and to the outer root sheath of the hair follicle [158]. $Ctsl^{-/-}$ mice developed epidermal hyperplasia, acanthosis and hyperkeratosis as a result of hyperproliferation of basal keratinocytes detected with the proliferating marker Ki67. In contrast, the Ki67 marker was not increased in $Ctsl^{-/-}$ mice expressing human cathepsin V, but remained the same as in wild-type mice. A further characteristic of $Ctsl^{-/-}$ mice was late morphogenesis and an altered hair follicle cycle. This, the disturbed cell death in the hair follicles and the disturbed differentiation of the inner root sheath led to periodic hair loss in

these mice. This characteristic was also almost completely abolished in cathepsin V-expressing $Ctsl^{-/-}$ mice [158].

Cathepsin L has also been shown to be important for the positive selection of CD4+ T cells; $Ctsl^{-/-}$ mice have a lower number of CD4+ T cells [62,160]. By introducing human cathepsin L or human cathepsin V into $Ctsl^{-/-}$ mice, Sevenich et al. were able to show that both human cathepsins can partially normalise the level of CD4+ T cells [160]. They performed experiments in two different types of mice: C57BL/6 mice in which the positive selection of CD4+ T cells depends on cathepsin L-mediated degradation of the MHC class II-associated invariant chain and proteolytic generation of positive selecting self-peptides, and FVB/N mice, in which the positive selection of CD4+ T cells is generally the result of the generation of positive selecting self-peptides. They concluded that the functional differences of cathepsin L and cathepsin V are determined by their integral biochemical properties and tissue-specific expression and that the invariant chain is a physiological substrate of cathepsin V [160]. Cathepsin V was further found to rescue the phenotype in $Ctsl^{-/-}$ mice with antigen-induced arthritis (AIA) [162]. In these mice the level of CD4+ T cells had normalised after human cathepsin V expression and consequently the effect of cathepsin L absence was abolished. Parameters like joint swelling, joint inflammation and destruction as well as serum levels of certain IgGs and some other cellular parameters were normalised to wild type levels [162]. Full or partial phenotype improvement was shown also in $Ctsl^{-/-}$ mice with progressive dilated cardiomyopathy. Here, epithelial expression of human cathepsin V completely rescued hair-loss, cardiac atrionatriuretic peptide expression and end systolic heart dimensions were significantly improved while there was no change in cardiac contraction nor heart weight [163]. Al-Hashimi and co-workers studied the role of N-terminally truncated cathepsin V in nucleus of thyroid epithelial cells carrying mutations, that in the end lead to a neoplastic phenotype [164]. Regarding mechanism of action, they have proposed that after overexpression of transcription factor E2F1 (it is overexpressed in thyroid cancer) it binds to the cathepsin V promoter and promotes the expression of cathepsin V as full-length and N-terminally truncated form. The truncated form enters the nucleus and triggers thyroid cell proliferation inciting carcinogenesis [164]. In a previous study the same group found similar action of cathepsin L in colorectal carcinoma cells where it accelerated cell cycle progression [165]. Bach et al., however, showed the same role for cathepsin D in breast cancer [166]. Any possible compensational role between cathepsins L and V in the nucleus and potentially between cathepsins V and D needs to be further examined. A later study, on the contrary, found in the nucleus pro and mature form of

cathepsin V but no N-terminally truncated one [167]. The presence of bipartite nuclear localisation sequence (NLS) in cathepsin V gene was demonstrated. Cathepsin L, however, transfers to the nucleus together with the transcription factor Snail [168]. Cathepsin V translocates to nucleus during the S phase of the cell cycle. Its depletion slows down the cycle and cells accumulate in the G2/M phase [167].

10. Cathepsins C and H

Cathepsins C (also known as dipeptidyl peptidase I) and H are the only aminopeptidases in the group of cysteine cathepsins. Additionally, cathepsin H cleaves substrates as an endopeptidase, depending on its localisation and the pH of the environment [1,169]. Cathepsin C is unique among the cysteine cathepsins in that it forms a 200 kDa homotetramer post-translationally [170,171]. It is widely distributed in many tissues where it is involved in non-specific intracellular protein degradation and plays the central role in the zymogen activation of serine peptidases in immune cells, including the activation of granzymes A and B, which are mediators of the granule-dependent cytotoxic pathway in NK cells and CTLs [172–174]. Cathepsin C is essential for granzyme A activation. However, for granzyme B activation the presence of an enzyme that compensates for its activity in after granule packaging was observed in $Ctsc^{-/-}$ mice with significant residual granzyme B activity and perforin-dependent cytotoxicity observed regardless of cathepsin C deletion [175]. To further investigate the activation of progranzyme B, cathepsin H was recognised as an additional granzyme B convertase in addition to cathepsin C (Figure 1, Table I) [176]. The redundant activity of both enzymes for progranzyme B activation was demonstrated in mice lacking both cathepsin C and H, which exhibited significantly reduced granzyme B activity in lymphocytes compared to mice lacking only cathepsin C. However, mice lacking both cathepsin C and H retain some granzyme B activity, indicating the presence of an additional granzyme B convertase in cytotoxic lymphocytes [176].

Cathepsin H is also a ubiquitously expressed cysteine cathepsin with diverse functions in physiological and pathological processes (reviewed in [177]). Structurally, cathepsin H differs from other cysteine cathepsins by the presence of an octapeptide mini-chain derived from propetide. Mini-chain is bonded to the body of the enzyme with disulphide bond and restricts access of larger substrates to the active site. In addition, the presence of a C-terminal carboxy group that contributes to docking of the positively charged N-terminal site of the proteins and leads to the aminopeptidase activity of cathepsin H [169,178]. However, a shift in the position of the mini-chain may occur, leading to endopeptidase activity of cathepsin H [179]. Among

other functions, cathepsin H is involved in the processing and secretion of pulmonary surfactant proteins [180,181]. However, studies have shown that cathepsin H deficiency does not reproduce the severe phenotype of mice with surfactant proteins deficiency, suggesting that other peptidases are involved in the secretory pathways [181–183]. Besides cathepsin H, cathepsin C is the only cysteine cathepsin that is colocalised in the secretory compartment of alveolar type II pneumocytes that process surfactant proteins [181–183]. Using $Ctsh^{-/-}$ mice, it was shown that the constitutive loss of cathepsin H in type II pneumocytes can be functionally compensated by cathepsin C or the aspartic peptidase cathepsin E [183]. Similar conclusions were also drawn in an earlier study by Yayoi et al. in which it was shown that both cathepsins C and H are co-localised in lysosomes of type II alveolar epithelial cells in the rat lung and participate in the process of surfactant formation, which contains specific proteins [184].

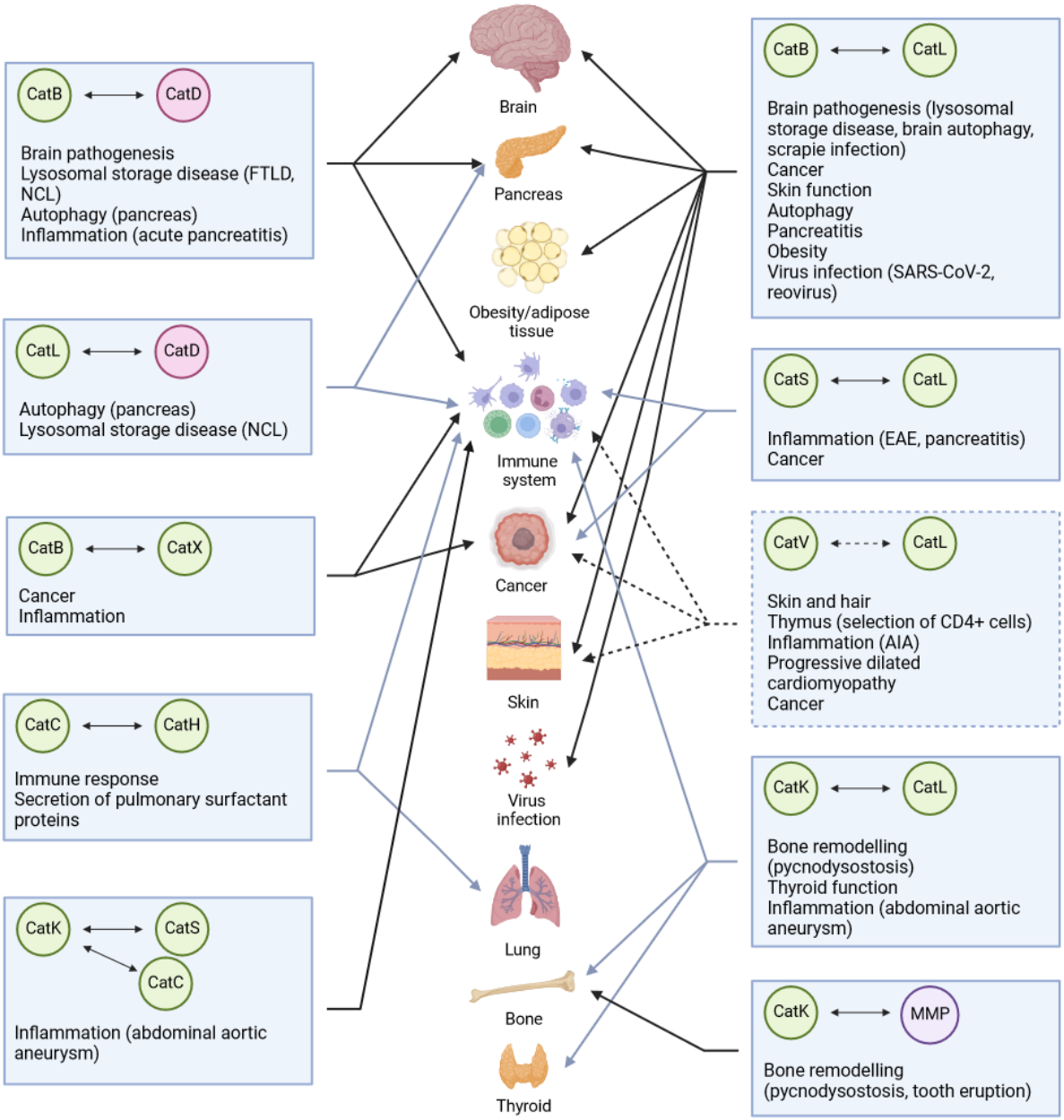

Figure 2. Graphical overview on the highlighted compensations between different cathepsins - association with pathologies and/or specific organs/tissues. Cathepsin (Cat), matrix metalloprotease (MMP), frontotemporal lobar degradation (FTLD), neuronal ceroid lipofuscinosis (NCL), antigen-induced arthritis (AIA). CatV/CatL are marked with dashed line as no actual compensation has been shown yet; experiments were done on Ctsl$^{-/-}$ mice with expressed human cathepsin V (see text). Figure was created using BioRender.com.

11. Conclusion

Cathepsins function within the complex proteolytic network where multidirectional interactions between different cathepsins have been observed. Changes in their activity and expression have been associated with a number of pathological processes including cancer, inflammation, neurodegenerative disorders, bone remodeling and other conditions (Figure 2). A number of evidences accumulated over the years on compensatory mechanisms and redundant function between different cathepsins in order to maintain tissue integrity and balance between different cathepsins. This indicates the importance of understanding cathepsin activity, their mechanisms of action and their interaction with each other and with other enzymes. This review on compensatory mechanisms between cathepsins highlights their role in the proteolytic network that regulate physiological and pathological processes in the absence of a particular enzyme. It is evident that compensation between cathepsins depends first on their specific activity, as compensation between carboypeptidases (cathepsins B and X) and aminopeptidases (H and C) is most usual, however, the compensation may occur also between different endopeptidases (cathepsins B, K, L, S, V). Nevertheless, the interaction between cathepsins and their function within networks is not easy to define, and in many cases, it is not clear whether two cathepsins have a redundant or compensatory function. Therefore, further discussion and research is needed to fully understand the relationships within complex proteolytic networks.

Cathepsins are promising therapeutic targets, and the complexity of their network with the presence of multiple compensational mechanisms suggests that their specific inhibitors need to be used as therapeutic agents with care to avoid unwanted side effects and that the inhibition of multiple cathepsins is necessary to successfully regulate proteolytic activity and control pathological processes.

Competing interest statement

The authors have no competing interests to declare.

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Author contribution

UPF and AM designed and wrote the manuscript. JK wrote and revised the manuscript. All authors approved the final version.

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Table I. Compensation between different cathepsins.

Cathepsin	Compensation partner	Pathology [substrate]	References
CatB	CatX	Cancer [ECM, profilin-1, integrin receptors, CXCL-12 chemokine, angiotensin 1]	[17,38,39,41,42]
		Inflammation (delayed-type hypersensitivity reaction) [antigen processing - invariant chain (Ii), MHC class II molecules]	[43]
CatB	CatD	Brain pathogenesis (cathepsin D deficiency) [α-synuclein]	[45,47,50]
		Lysosomal storage disease (frontotemporal lobar degradation (FTLD) and neuronal ceroid lipofuscinosis (NCL)) [progranulin]	[59]
		Autophagy (pancreas)	[51,57]
		Inflammation (acute pancreatitis) [trypsinogen]	[52]
CatD	CatL	Autophagy (pancreas)	[51,57]
		Lysosomal storage disease (neuronal ceroid lipofuscinosis (NCL)) [saponin C]	[60]
CatB	CatL	Brain pathogenesis (lysosomal storage disease, brain atrophy) [lysosomal enzymes, molecules associated with ubiquitin-conjugating system, apolipoprotein E, neurochondrin, Rab14, carboxypeptidase E, calcyon, Delta/Notch-like epidermal growth factor-related receptor (DNER)]	[58,66–68,70]
		Scrapie infection [Prion proteins (PrP^{Sc})]	[71]
		Cancer [ECM, signalling molecules]	[68,76]
		Skin function [matricellular proteins, ECM, signalling proteins]	[68,70]
		Autophagy	[66]

		Pancreatitis [trypsinogen]	[73–75]
		Obesity [adipose tissue]	[78]
		SARS-CoV-2 virus infection [spike protein]	[79,84–91]
		Reovirus infection [viral capsid proteins]	[93]
CatS	CatL	Inflammation	
		Antigen presentation [antigen processing]	[99]
		Cancer-related inflammation [invariant chain (Ii), MHC class II molecules]	[62,100–105,107]
		Neuroinflamation [Ii chain]	[106]
		Autoimmune encephalomyelitis (EAE) [MHC class II molecules, antigen processing]	[98]
		Pancreatitis [trypsinogen]	[112]
		Cancer [pro-cathepsin C, endostatin]	[107,110]
CatK	CatL	Bone remodelling (pycnodysostosis) [collagen]	[120,126]
		Thyroid function [thyroglobulin prohormone]	[129,131,132]
		Inflammation (abdominal aortic aneurysm) [ECM]	[133,139]
CatK	CatS, CatC	Inflammation (abdominal aortic aneurysm) [ECM]	[138]
CatK	MMPs	Bone remodelling [collagen]	
		Pycnodysostosis [collagen]	[120,123,143,144,150,151]
		Tooth eruption	[155–157]

		[bone matrix proteins, connective tissue or lamina propria, ECM]	
CatV*	CatL*	Skin and hair	[158]
		Thymus (selection of CD4+ T cells)	[160]
		[invariant chain (Ii), MHC class II molecules]	
		Inflammation	
		Antigen induces arthritis (AIA)	[162]
		Progressive dilated cardiomyopathy	[163]
		Cancer	[167]
		[effect on cell cycle]	
CatC	CatH	Immune response (granule-dependant cytotoxic pathway – cytotoxicity of NK cells and CTLs)	[176]
		[progranzyme A and B]	
		Secretion of pulmonary surfactant proteins	[181–184]
		[surfactant proteins]	

Cat – cathepsin, ECM – extra cellular matrix, NK – natural killer cells, CTLs – cytotoxic T lymphocytes

* CatV/CatL – no actual compensation has been shown yet; experiments were done on $Ctsl^{-/-}$ mice with expressed human cathepsin V (see text)