Lysosomal peptidases—intriguing roles in cancer progression and neurodegeneration

Janko Kos1,2, Ana Mitrović2, Milica Perišić Nanut2 and Anja Pišlar1

1 Faculty of Pharmacy, University of Ljubljana, Slovenia
2 Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia

Keywords
cancer; cathepsins; lysosomes; neurodegeneration; peptidases

Correspondence
J. Kos, University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia
E-mail: janko.kos@ffa.uni-lj.si

(Received 8 October 2021, revised 4 January 2022, accepted 20 January 2022)
doi:10.1002/2211-5463.13372

Lysosomal peptidases are hydrolytic enzymes capable of digesting waste proteins that are targeted to lysosomes via endocytosis and autophagy. Besides intracellular protein catabolism, they play more specific roles in several other cellular processes and pathologies, either within lysosomes, upon secretion into the cell cytoplasm or extracellular space, or bound to the plasma membrane. In cancer, lysosomal peptidases are generally associated with disease progression, as they participate in crucial processes leading to changes in cell morphology, signaling, migration, and invasion, and finally metastasis. However, they can also enhance the mechanisms resulting in cancer regression, such as apoptosis of tumor cells or antitumor immune responses. Lysosomal peptidases have also been identified as hallmarks of aging and neurodegeneration, playing roles in oxidative stress, mitochondrial dysfunction, abnormal intercellular communication, dysregulated trafficking, and the deposition of protein aggregates in neuronal cells. Furthermore, deficiencies in lysosomal peptidases may result in other pathological states, such as lysosomal storage disease. The aim of this review was to highlight the role of lysosomal peptidases in particular pathological processes of cancer and neurodegeneration and to address the potential of lysosomal peptidases in diagnosing and treating patients.

Lysosomes are membrane-bound organelles that are found in most cells. They were discovered and named by Christian de Duve (reviewed in [1]) and later recognized as the main waste disposal system of the cell, digesting both intracellular and extracellular materials [2]. Lysosomes have a diameter of 0.1–1.2 μm and a pH of 4.5–5.0 [3]. The two main pathways of waste entry into lysosomes are endocytosis and autophagy, which internalize extracellular and intracellular material, respectively.

During endocytosis, a part of the cell’s plasma membrane forms vesicles that embed extracellular material. These vesicles arise at the plasma membrane through a variety of mechanisms [4,5]. Clathrin-dependent endocytosis accounts for the formation of most endocytic vesicles. It involves binding between the clathrin and cytoplasmic domains of plasma membrane proteins, formation of clathrin-coated pits, and budding of clathrin-coated vesicles. Clathrin-coated vesicles are internalized and then fuse with specific acceptor

Abbreviations
Cat, cathepsin; CDK2-AP1, cyclin-dependent kinase 2-associated protein 1; CDP/Cux, CCAAT-displacement protein/cut homeobox; ECM, extracellular matrix; EGF, epithelial growth factor; EMT, epithelial–mesenchymal transition; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; IGF-I, insulin-like growth factor I; JNK, c-Jun N-terminal kinase; LRP1, low-density lipoprotein receptor-related protein 1; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemotactic protein 1; MDSCs, myeloid-derived suppressor cells; MMPs, matrix metallopeptidases; PAR4, proteinase-activated receptor 4; PI3K, phosphatidylinositol-bisphosphate 3-kinase; RIPK1, receptor-interacting Ser/Thr protein kinase 1; RPLP0, ribosomal protein P0; TGF-β, transforming growth factor-β; TIMPs, tissue inhibitors of metalloproteases; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.
compartments [6]. Conversely, clathrin-independent endocytosis comprises several internalization mechanisms, of which the most recognized are caveola-dependent [7] and actin-driven [8]. Furthermore, micropinocytosis is another mechanism of endocytosis that occurs in highly ruffled regions, forming actin extensions around extracellular fluid [9]. All endocytosed materials first reach early endosomes, in which they are sorted either for recycling or degradation. Transport toward lysosomes involves globular late endosome intermediates, also called multivesicular bodies [10].

Autophagy represents the uptake of intracellular components into endo/lysosomal compartments. Autophagy is divided into different types, including macroautophagy [11], microautophagy [12], chaperone-mediated autophagy [13], and various types of selective autophagy leading to the destruction of particular organelles.

Normal lysosomal function is enabled through the actions of two classes of proteins: lysosomal membrane proteins and lysosomal enzymes, predominantly soluble lysosomal hydrolases (also referred to as acid hydrolases). Approximately 50 lysosomal membrane proteins have been described so far. Soluble lysosomal hydrolases constitute the main degradative lysosomal component, and to date, more than 50 hydrolases and their accessory proteins have been detected. Lysosomal hydrolases comprise a variety of peptidases, nuclease, and glycosidases, sulfatases, and lipases [14]. The aim of this review was to highlight the role of lysosomal peptidases, in particular lysosomal cathepsins, in the pathological processes of cancer and neurodegeneration.

**Lysosomal peptidases**

Peptidases represent a large family of lysosomal hydrolases, which are present in all living organisms and catalyze the hydrolysis of peptide bonds in different biological processes. Almost 600 human peptidases are listed in the MEROPS database and can be classified into seven main groups according to their structure and catalytic type: serine, cysteine, threonine, and aspartyl, glutamyl, asparaginyl, and metallopeptidases [15]. Lysosomal peptidases are involved in the complete breakdown of proteins targeted to lysosomes; however, they may also have other regulatory functions either within or outside of lysosomes.

In mammalian cells, the most predominant lysosomal peptidases can be grouped into four major families: aspartic cathepsins (D and E), serine cathepsins (A and G), the asparagine endopeptidase legumain (a cysteine peptidase with a fold more related to caspasins than family C1 cathepsins [16]), and cysteine cathepsins (B, C, F, H, K, L, O, S, V, W, and X/Z), annotated as clan CA, family C1a [17].

The serine peptidase cathepsin (Cat) A is involved in regulating the activity of lysosomes, stability of lysosomal glycosidases (e.g., beta-D-galactosidase and N-acetyl-alpha-neuraminidase), and transport of neuraminidase to mature lysosomes [18]. In addition, this serine peptidase triggers the degradation of LAMP-2A, a receptor involved in chaperon-mediated autophagy [18].

Another serine peptidase, Cat G (CatG), is known as the main granule-associated proteolytic enzyme of neutrophils [19]. However, it is also found in the endo/lysosomal compartments of a variety of antigen-presenting cells, in which it plays a critical role in antigen and autoantigen processing [20]. For example, CatG is involved in proteolytic cleavage, subsequent activation of chemokines, cytokines, and cell surface receptors, antigen processing, and clearance of internalized pathogens [21–23]. As such, CatG is involved in the defense against invading pathogens. However, its dysregulated proteolytic activity contributes to pathological conditions such as chronic pulmonary diseases, human immunodeficiency virus infection, tumor progression, and metastasis (extensively reviewed in [20,24]).

Cathepsin E (CatE) is an aspartic peptidase and a member of the A1 peptidase family, along with pepsin A and Cat D (CatD). CatE is expressed intracellularly in gastrointestinal cells, immune cells, and several cell types within lymphoid tissues [25,26]. In antigen-presenting cells, CatE is located in endo/lysosomal compartments and is most likely involved in the breakdown of antigenic proteins [27]. However, in erythrocytes and gastric cells, CatE is expressed in the plasma membrane [27–29], and its function is not fully understood. Furthermore, a recent study has shown it may be an early biomarker for certain types of cancer [25].

The most abundant aspartic lysosomal peptidase is CatD [30]. Through its endopeptidase activity, CatD modulates the activity of diverse polypeptides, growth factors, and enzymes and the degradation of misfolded, long-lived, and denatured proteins. Accordingly, CatD is considered an essential regulator of cell signaling and cellular homeostasis [31]. Dysregulated CatD activity plays an important role in diseases such as acute kidney injury, coronary events, neurodegenerative diseases, and cancer (reviewed in [31–34]).

Legumain (also named asparagine endopeptidase; clan CD, family C13) is named after its strict specificity for cleavage of asparagine residues and was classified as a member of the C13 family of cysteine peptidases (EC 3.4.22.34) [35]. Legumain is predominantly located...
in late endo/lysosomes of antigen-presenting cells, such as B cells and dendritic cells. In B cells, legumain participates in processing endogenous and foreign proteins for presentation of the major histocompatibility complex class II molecules on the surface of T cells [36], whereas in dendritic cells, legumain plays an indispensable role in activating Toll-like receptor (TLR) 9, which is critical for full cytokine production [37]. Legumain was reported to regulate the stability of FOXP3, a transcription factor that controls the immunosuppressive program in CD4⁺ T cells [38]. It also plays a role in osteoclast formation and bone resorption (by regulating the differentiation fate of human bone marrow stromal cells) and in extracellular matrix (ECM) remodeling in kidneys, lung, liver, and pancreas (reviewed in [16]). Finally, dysregulated legumain activity is associated with cancer and neurodegenerative diseases, including Alzheimer’s disease (AD), stroke, ischemia, amyotrophic lateral sclerosis (ALS), and multiple sclerosis [39,40].

The fourth family consists of papain-like cysteine peptidases, which are the main focus of this review. They represent the largest family of cathepsins, with 11 cysteine cathepsins encoded in the human genome (B, C/DPP1, F, H, K, L, O, S, W, V, and X/Z). Some cysteine cathepsins, such as cathepsins B, H, and L, are ubiquitously expressed in human tissues and represent enzymes with broad substrate specificities. However, certain cysteine cathepsins (e.g., S, X, V, K, and W) are strictly expressed in specific cell types (reviewed in [41]). Most of them exhibit endopeptidase activity (by cleaving internal peptide bonds), whereas only a few exhibit exopeptidase activity and possess additional structural elements that restrict access to the active site and form electrostatic bonds with the C or N termini of substrates [42,43]. Due to these structural variances, cathepsins B (CatB) and X (CatX; also known as Cat Z, P, IV/B2/Y, and lysosomal carboxypeptidase B) can act as dipeptidyl carboxypeptidases and carboxymonopeptidases, respectively [44,45], whereas cathepsins C (CatC; also known as dipeptidyl peptidase I) and H (CatH) cleave their substrates as aminopeptidases [15,46]. Only CatB and CatH exhibit both endopeptidase and exopeptidase activities, depending on their localization, that is, the pH of the environment [47,48].

In specialized immune cells, such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, several other peptidases can be found in the endo/lysosomal pathway. These cells contain secretory lysosomes, that is, cytotoxic granules, which are exocytosed during specific interaction with target cells. Cytotoxic granules contain serine peptidase granzymes and perforin, which, together with cysteine cathepsins, trigger apoptosis in target cells [49].

The activity of cathepsins is controlled by different mechanisms, which include peptidase expression (regulated at the transcriptional and translational levels), cofactors, lysosomal trafficking, the specificity of the active site cleft, and pH. Furthermore, cathepsins are synthesized and delivered to early lysosomes as inactive precursors, which are further activated either by lower pH, proteolytic processing by other endo/lysosomal hydrolases, or interaction with glycosaminoglycans [50–56]. Cathepsin activity was examined in various kinetic studies using specific substrates and visualized by fluorescently labeled activity-based probes both in vitro and in vivo [57–60]. Ultimately, endogenous protein inhibitors regulate the activity of mature cathepsins that escape endo/lysosomal vesicles and are present in the cytoplasm or extracellular space or bound to the plasma membrane. Several groups of endogenous inhibitors of cysteine, serine, and metallopeptidases have been shown to impair secreted or misdirected lysosomal cathepsins, including cystatins, serpins, and tissue inhibitors of metallopeptidases [61,62]. Nevertheless, certain exceptions, such as cystatins M and F, can enter the endo/lysosomal pathway and regulate intralysosomal peptidase activity [62].

Lysosomal cathepsins were long believed to participate in only intracellular protein turnover; however, later, they were found to also play roles in a number of other physiological and pathological processes [63]. For example, extralysosomal cathepsins have been associated with prohormone activation, apoptosis, cell migration, cancer, and neurodegeneration [64,65].

Lysosomal peptidases in cancer progression

**Lysosomal peptidases in cancer cell signaling**

The development and progression of cancer is a complex multistep process of genetic and epigenetic alterations that drive the transformation of normal cells to their malignant forms [66,67]. The transformation is often followed by dysregulation of lysosomal proteolytic enzymes involved in signaling pathways in transformed or malignant cells [50,68,69]. Lysosomal peptidases interfere with cytokine/chemokine signaling and modify growth factors and receptors crucial for tumor cell growth and proliferation (Table 1; reviewed in [68,70]). CatB participates in the signaling of activators of proliferating cells, such as insulin-like growth factor I (IGF-I) and transforming growth factor-β (TGF-β). IGF-I is important for regulating and
**Table 1. Significance of lysosomal peptidases in cancer.**

| Type of Cat | Function | Role in cancer | References |
|-------------|----------|----------------|------------|
| CatB        | IGF-I, EGF, TGF-β signaling | Tumor cell growth, cell proliferation, invasion, EMT, angiogenesis | [71,73,74,77,78,142] |
|             | Regulation of MAPK/ERK and PI3K/Akt signaling, TLR3 | Tumor progression | [79–81,141] |
|             | Cleavage of cell cycle inhibitor p27Kip1 | Tumor cell proliferation | [82] |
|             | Cleavage of Bid, Bcl-2 family proteins, lipid signaling enzyme sphingosine kinase 1, RIPK1 | Apoptosis, cell death | [124–126,128–130] |
|             | Degradation of ECM proteins | Invasion, metastasis, angiogenesis | Reviewed in [56] |
|             | Induction of EMT, regulation of EMT markers | EMT, invasion | [142,143,145] |
|             | Degradation of angiogenesis inhibitors and release of growth factors | Angiogenesis | [75,182,244] |
|             | MDSC function and activity | Tumor progression, suppression of antitumor immunity | [235] |
| CatL        | EGF, TGF-β processing and signaling | Tumor cell growth, EMT, invasion | [83,147] |
|             | Activation of MAPK/ERK and PI3K/Akt signaling CDP/Cux and 53DP1 processing | Angiogenesis, EMT, invasion | [84,85,148,149] |
|             | Cleavage of Bid, Bcl-2 family proteins, complement and CDK2-AP1 | Tumor cell proliferation, EMT, angiogenesis | [84,88,91–93] |
|             | Degradation of ECM proteins | Apoptosis, cell proliferation | [86,125,126,131,215] |
|             | Induction of EMT, regulation of EMT markers | Invasion, metastasis, angiogenesis | Reviewed in [90,146] |
|             | Release of growth factors, endothelial cell infiltration | Angiogenesis | [163] |
|             | C-terminal processing of perforin | Granzyme-mediated apoptosis | [197] |
|             | Th17 subset and MDSC differentiation | Suppression of antitumor immunity | [219,233] |
| CatV        | Suppression of GATA3 expression | Hyperproliferation | [94,95] |
|             | Regulation of epithelial and mesenchymal markers | EMT, invasion | [152] |
| CatS        | Elastin degradation | Invasion, metastasis | [153] |
|             | Regulation of PI3K/Akt/mTOR, JNK, ERK/MAPK, TGF-β signaling | Autophagy, EMT, invasion | [97,99,156] |
|             | Cleavage of Bid, Bcl-2 family proteins, RIPK1, CD74 | Apoptosis | [125–127,129,243] |
|             | Degradation of ECM proteins | Invasion, metastasis, angiogenesis | [154] |
|             | Regulation of EMT markers | Angiogenesis | [145,155] |
|             | Generation of anti- and proangiogenic peptides | Suppression of antitumor immunity | [184,154] |
|             | Th17 subset differentiation | EMT, invasion | [152] |
| CatK        | Regulation of TLR, Notch signaling, cytokines | Tumor progression, cellular crosstalk, inflammation | [100,101,238,240,241] |
|             | Reviewed in [158] | | |
|             | Cleavage of Bid, Bcl-2 family proteins | Apoptosis | [125,126] |
|             | Degradation of ECM proteins, resorption of bone matrix | Invasion, metastasis, angiogenesis | Reviewed in [146,158] [104] |
| CatH        | Cleavage of Bid, Bcl-2 family proteins | Apoptosis | [125,126] |
|             | Talin processing, functional development of tumor vasculature | Migration, adhesion, angiogenesis | [157,185] |
|             | N-terminal processing and activation of granzymes | Granzyme-mediated apoptosis | [200] |
| CatC        | Interaction with TNF-α/p38 MAPK, JNK signaling | Cell proliferation, metastasis, apoptosis, autophagy | [105,133] |
|             | N-terminal processing and activation of granzymes | Granzyme-mediated apoptosis | [199] |
| CatX        | Regulation of MAPK/ERK, PI3K/Akt, FAK/Src, IGF-I signaling, RPLP0 | Tumor progression, migration, apoptosis | [68,106,108,134] |
maintaining the invasive and metastatic properties of the malignant phenotype [71], whereas TGF-β exhibits both tumor-suppressive and tumor-promotive properties [72] and is a key regulator of the epithelial–mesenchymal transition (EMT). CatB regulates the production and signaling of TGF-β by direct activation [73,74] or by ECM proteolysis and subsequent TGF-β release [75]. The downregulation of CatB (both by silencing and inhibition) reduces TGF-β signaling and invasion [73,76]. CatB is also responsible for the degradation of epithelial growth factor (EGF) and its internalized receptor complex, as observed in thyroid cancer, glioma cells, and liver [77,78].

Furthermore, CatB mediates tumor progression by regulating kinases involved in Ras/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling. Loss of CatB was shown to downregulate the MAPK/ERK pathway in pancreatic cancer [79]. Similarly, in glioma cells, CatB regulates cell migration through c-Jun N-terminal kinase (JNK), another member of the MAPK family [80]. CatB also regulates phosphatidylinositol-bisphosphate 3-kinase (PI3K)/Akt signaling, another pathway that is crucial for tumor progression. Reduced activation of PI3K/Akt signaling was demonstrated in gliomas after CatB downregulation [81]. CatB also promotes tumor cell proliferation by cleaving cell cycle inhibitor p27Kip1; higher p27Kip1 levels, followed by increased cyclin B1 levels, were observed in CatB-deficient colorectal tumors [82].

Another lysosomal cysteine Cat involved in cancer cell signaling is Cat L (CatL). During tumor growth, it is responsible for cleaving EGF receptor and consequently activating downstream signaling pathways [68,83]. Interestingly, CatL-deficient mouse keratinocytes exhibited increased activation of MAPK/ERK and PI3K/Akt

| Table 1. (Continued). |
|-------------------|----------------------------------|----------------------------------|-----------------------------|
| Type of Cat       | Function                          | Role in cancer                   | References                  |
| Integrin receptor signaling, cleavage of profilin-1 | Adhesion, migration               | Reviewed in [107], [163,164]      |
| Compensation of CatB proteolytic activity | Invasion, metastasis              | [163,164]                         |
| Bypassing senescence | Tumor progression                 | [165]                            |
| Induction of EMT, regulation of EMT markers | EMT, invasion, migration          | [142,166]                         |
| MDSC differentiation | Suppression of antitumor immunity tumor progression | [233]                            |
| Legumain | Regulation of PI3K/Akt, MYC, p53 signaling | Cell proliferation, apoptosis | [109,110,373] |
| Interaction with integrin receptors | Cell proliferation, metastasis, migration, EMT | [111–113]                         |
| Producing the mature forms of MMPs and cathepsins | Invasion, metastasis, angiogenesis | [168,169]                         |
| Induction of EMT, regulation of EMT markers | EMT, invasion                     | [170]                            |
| Processing of CatL and Th17 subset differentiation | Suppression of antitumor immunity | [217]                            |
| CatD | Interaction with IGF-II receptor, LRP1-regulated intermembrane proteolysis | Tumor cell growth, cell proliferation | [115,118] |
| Regulation of ERK and PI3K/Akt in signaling | Tumor cell proliferation, migration, angiogenesis | [116,117]                         |
| Activation of Bix, caspase-8 | Apoptosis                         | [114,135]                         |
| Affecting the fusion of autophagosomes and lysosomes | Apoptosis, autophagy               | [137]                            |
| Degradation of ECM proteins | Invasion, metastasis, angiogenesis | [114]                            |
| Cleavage of peptidases and their endogenous inhibitors | Invasion, metastasis               | [75,171–173]                      |
| Releasing growth factors from the ECM proteins and degradation of antiangiogenic factors | Angiogenesis                      | [114,187,188]                     |
| CatG | IGF-1, TGF-β signaling | Tumor cell growth, bone resorption, angiogenesis, cell aggregation | [119–121] |
| Downregulation of survivin expression | Apoptosis                          | [138]                            |
| Regulation of VEGF, MCP-1, PAR4, cell surface proteins | Invasion, migration, angiogenesis | [178,180,190]                     |
| CatE | Induction of EMT, regulation of EMT markers | EMT                               | [175]                        |
| Release of soluble TRAIL | Growth arrest, apoptosis            | [176]                            |
| Upregulation of antiangiogenic mediators | Angiogenesis                       | [189]                            |
signaling pathways and elevated levels of active Ras [84]. Ras is one of the central molecules in several cancer-promoting signaling pathways, such as MAPK and Akt [84]. In human omental microvascular endothelial cells, CatL activated the ERK pathway and induced angiogenesis [85]. During cell cycle progression, CatL interacts with cell cycle regulator cyclin-dependent kinase 2-associated protein 1 [86], a growth suppressor that negatively regulates cyclin-dependent kinase 2 [87]. In cancer cells, CatL is also localized in the nucleus. Nuclear CatL processes the CCAAT-displacement protein/cut homeobox (CDP/Cux) transcription factor to enhance DNA binding [88,89]. CDP/Cux promotes tumor cell proliferation by accelerating cell entry into the S phase of the cell cycle and induces EMT by upregulating Snail, Slug, and E-cadherin promoters [90,91]. CatL-induced CUX1 activation may also contribute to triple-negative breast cancer via estrogen receptor-α repression [92]. Additionally, nuclear CatL involves CDP/Cux-independent mechanisms of tumor promotion. In triple-negative breast cancer, loss of BRCA1 activates nuclear CatL-mediated p53-binding protein 1 degradation, which acts as a replacement of BRCA1 that bypasses growth arrest and increases the survival of tumor cells. Moreover, this process activates DNA repair, which leads to increased therapy resistance [93]. The highly related CatL analogue, Cat V (CatV), also localizes to the nucleus in tumor cells, triggering hyperproliferation [94]. In breast cancer, nuclear CatV suppresses the expression of GATA3, a member of the zinc finger transcription factor family, by facilitating its turnover via proteasomes [95].

Cathepsin S (CatS) contributes to cancer progression by mediating autophagy. In human glioblastoma cells, CatS inhibition induces autophagy and an intrinsic pathway of apoptosis due to the production of reactive oxygen species [96–98] and subsequent suppression of PI3K/AKT/mTOR signaling and activation of JNK signaling [97]. Furthermore, CatS inhibition could also induce autophagy by activating the EGF receptor-related ERK/MAPK signaling pathway [99].

Cathepsin K (CatK) is mainly implicated in bone matrix resorption; however, it modulates cancer signaling by regulating the TLR and Notch pathways [100,101]. Additionally, CatK may regulate cytokines that are relevant for cellular crosstalk [102–104]. Furthermore, CatC promotes proliferation and metastasis in hepatocellular carcinoma by interacting with the tumor necrosis factor α (TNF-α)/p38 MAPK signaling pathway [105].

CatX also interferes with key signaling pathways, facilitating IGF signaling and affecting downstream signaling through focal adhesion kinase (FAK) [106]. CatX is also involved in the MAPK/ERK and PI3K/Akt signaling pathways [68]. Additionally, multiple studies demonstrated that CatX importantly contributes to tumor cell signal transduction due to its interaction with integrin receptors [107]. Through integrin-mediated pathways, CatX interacts with the FAK/Src signaling pathway and dysregulates cell migration [108].

Legumain promotes tumor progression via the PI3K/Akt signaling pathway [16] and cleaves tumor suppressor p53 in glioblastoma cells [109]. Additionally, inhibition of the p53 pathway and activation of the MYC pathway by legumain secreted from glioblastoma cells lead to the malignant transformation of normal astrocytes, which enhances the invasive ability of glioblastoma cells [110]. Tumor-derived legumain interacts with endothelial integrin αvβ3 through its Arg-Gly-Asp (RGD) motif and indirectly downregulates the expression of zonula occludens 1 via the STAT3 signaling pathway, which can promote tumor metastasis by increasing the permeability of endothelial barriers [111,112]. Moreover, in ovarian carcinoma cells, legumain interacts with integrin α5β1 and forms complexes that are secreted. These complexes are internalized by peritoneal mesothelial cells, in which they promote proliferation and migration via the FAK/Akt/ERK signaling pathways and contribute to EMT [113].

Both the proform and mature forms of CatD enhance cancer progression through both proteolytically dependent and independent manners [114]. Pro-CatD acts as a protein ligand and stimulates the proliferation of breast cancer cells through an autocrine mechanism. It interacts with the IGF-II receptor and can bind to the M6P/IGF-II receptor on the surface of breast cancer cells [115]. Both CatD and pro-CatD also promote cancer proliferation and migration by inducing phosphorylation of ERK and PI3K/Akt through a nonproteolytic mechanism [116,117]. Moreover, it induces the outgrowth of fibroblasts by binding to the receptor of the low-density lipoprotein receptor-related protein 1, thus inhibiting intermembrane proteolysis that is regulated by this protein [118].

CatG interacts with oncogenic proteins from the TGF-β pathway. It promotes TGF-β signaling at the tumor–bone interface by proteolytic activation of matrix metalloproteinase 9 (MMP9), thus promoting tumor growth and enhancing osteoclast activation and subsequent bone resorption [119]. Furthermore, CatG also triggers the IGF-I signaling pathway, which is partly responsible for cell aggregation [120]. Recently, it was demonstrated that CatG induces continuous phosphorylation of IGF-1R and Akt, indicating that CatG-specific IGF-I increases are caused by digestion of the IGF-binding protein IGFBP-2, and not IGF-I [121].
Lysosomal cathepsins also participate in regulating apoptosis [122,123]. Cathepsins B, L, S, H, and K, but not cathepsins C and X, act proapoptotically by activating Bid to tBid, which activates caspases [124], and antiapoptotically by cleaving and inactivating members of the Bcl-2 family [124–127]. Moreover, CatB suppresses alternative forms of cell death by cleaving the lipid signaling enzyme sphingosine kinase 1 [128] and degrading receptor-interacting Ser/Thr protein kinase 1 (RIPK1), consequently enforcing apoptosis [69,129]. Recently, CatB was identified as an executor of ferroptosis, a necrotic form of cell death caused by inactivation of the glutathione system and uncontrolled iron-mediated lipid peroxidation. [130]. By inhibiting complement-mediated tumor cell death, CatL promotes tumor growth and survival of melanoma cells [131]. Next, inhibition of CatS was suggested to upregulate the expression of the proapoptotic Bim protein at post-translation levels independent of the AMP-activated protein kinase and MAPK signaling pathways [132]. In addition to CatB, CatS also degrades RIPK1 and suppresses necroptosis [69,129].

In combination with other cathepsins, CatC is also involved in autophagic turnover by inducing endoplasmic reticulum stress and apoptosis mediated by JNK signaling [133]. By interacting with ribosomal protein P0 in the cytoplasm, CatX dysregulates apoptotic signaling and promotes tumor progression, as the knockdown of CatX led to G1 cell cycle arrest and apoptosis [134].

An important role in regulating apoptosis has also been demonstrated for CatD. It acts proapoptotically by activating Bax (directly or indirectly) [70,114] and caspase-8 [135]. Conversely, CatD can function as an antiapoptotic mediator of autophagy to protect cells under stress [136]. Recently, CatD inhibition was demonstrated to enhance the radiosensitivity of glioblastoma cells by attenuating autophagy through its effects on the fusion of autophagosomes and lysosomes [137]. In apoptosis, CatG downregulates survivin expression at the post-translational level via 5-lipoxygenase-mediated reactive oxygen species production and significantly blocks TNF-related apoptosis-inducing ligand-induced apoptosis [138].

**Lysosomal peptidases in invasion, migration, and metastasis**

In general, lysosomal peptidases can promote tumor invasion, cell migration, and metastases directly by degrading ECM proteins or indirectly by cleaving other factors within proteolytic cascades (Table 1; reviewed in [16,70,139,140]). The best studied lysosomal peptidase involved in these processes is CatB. It contributes to tumor progression by directly degrading ECM proteins such as laminin, fibronectin, collagen types I and IV, and proteoglycans. Additionally, CatB indirectly participates in degrading ECM by activating other peptidases that degrade ECM (reviewed in [56]). CatB also promotes cell migration by activating TLR 3 [141].

Furthermore, CatB promotes tumor progression by inducing EMT in which higher CatB protein levels are linked with a more invasive mesenchymal cell phenotype (Fig. 1A) [142] and with EMT activators through the E-box element in the CatB promoter [143]. E-cadherin, a cell membrane protein and a component of adherens junctions, whose inactivation is a key event during EMT [144], has also been identified as a substrate of CatB [145].

Similar to CatB, CatL directly degrades proteins of the ECM and basal membrane (e.g., laminin, fibronectin, collagen types I and IV, and elastin) or activates other peptidases in proteolytic cascades (reviewed in [90,146]). Moreover, extracellular CatL promotes tumor cell invasion via EMT by degrading E-cadherin and other adhesion proteins [145]. Downregulation of CatL inhibits TGF-β-induced EMT and cancer cell invasion and migration [147]. It suppresses EMT-inducing transcription factor Snail, which is associated with the PI3K/Akt and Wnt signaling pathways [148,149]. CatL-induced EMT through the Akt/glycogen synthase kinase-3β/Snail pathway was demonstrated in glioma cells [149]. Several studies demonstrated that the regulatory effects of CatL on the EMT are attributed to the proteolytic processing of the transcription factor CUX1 [148,149]. CatL induced by transcription factors (e.g., forkhead box O3A or K-ras) or ionizing radiation was shown to play a crucial role in EMT [148,150]. CatL is also involved in EMT by regulating RhoA and CDC42 signaling in vitro and in vivo [151].

Another Cat involved in EMT is CatV, as it increases levels of activated urokinase-type plasminogen activator and alters the expression of proteins associated with EMT [152]. Among human cathepsins, CatV has the most potent elastolytic activity and is particularly important in intracellular elastin degradation in macrophages [153]. Additionally, CatS contributes to the degradation of ECM [154]. Its preinvasive function could be explained by its ability to cleave cell adhesion proteins, including E-cadherin [145] and junctional adhesion molecule B [155]. Inhibition of CatS can reverse TGF-β-induced EMT, restore TGF-β-induced tight junction protein turnover, and consequently decrease the mobility of glioblastoma cells [156]. Furthermore, CatH regulates the migration...
of prostate cancer cells by processing talin (which affects integrin activation and adhesion) [157]. Additionally, CatK, which is predominantly extracellularly located, contributes to tumor progression by degrading collagen types I and II, elastin, vascular endothelial factor (VEGF), adiponectin, aggrecan, and osteonec-tin. Moreover, CatK is the Cat with the most efficient collagenase activity (reviewed in [146,158]). In connection with other peptidases (e.g., MMPs), CatK cleaves stromal cell-derived factor 1α, osteopontin, and stem cell factor, thereby altering cell signaling and enabling the release of stem cells into the ECM [158,159].

Carboxyolopeptidase CatX contributes to tumor progression through mechanisms other than ECM degradation. Multiple substrates of CatX have been identified, including the β-chain of integrin receptors, γ-enolase, chemokine CXCL-12, bradykinin, kallidin, huntingtin, and profilin-1 (reviewed in [107]). CatX enhances migratory and invasive properties of tumor cells by interacting with integrin receptors and profilin-1 [160–162] and promotes tumor progression by compensating CatB proteolytic activity [163,164], bypassing senescence [165], and inducing EMT [142,166]. In EMT, higher CatX levels correlate with an increased expression of mesenchymal markers (Fig. 1A) and decreased expression of epithelial markers. Overexpression of CatX during EMT is also associated with upregulation of MMP2, MMP3, and MMP9, which enable the remodeling of the ECM [142,166].

In addition to its intracellular activity, legumain can be secreted into the tumor microenvironment (TME) in which it contributes to degrading and remodeling the ECM [16,167], either by producing the mature forms of MMP2 and MMP9 [16,168] or by processing cathepsins [169]. In gastric carcinoma, legumain knockdown resulted in changes in downstream EMT signaling pathways (e.g., Twist), with increased E-cadherin and decreased mesenchymal markers [170]. CatD promotes tumor invasion, migration, and...
Peptidases in cancer and neurodegeneration

J. Kos et al.

metastasis by cleaving ECM proteins, cytokines, and chemokines locally or by nonproteolytic mechanisms [114]. By activating CatB, CatD is capable of triggering processes downstream of the proteolytic cascades [75]. Next, proteolytically active CatD stimulates the activity of secreted plasminogen activators by degrading plasminogen activator inhibitor-1 [171]. CatD was also observed to cleave endogenous inhibitors of cysteine peptidases: stefin B and cystatin C [172,173]. Moreover, a recent study demonstrated the involvement of CatD and its proform in the migration of mesenchymal stem cells to tumor sites [174].

In contrast to most other cathepsins, higher CatE levels are associated with improved survival of cancer patients (reviewed in [70]). Deletion of CatE in mice resulted in the spontaneous development of mammary tumors and was associated with EMT and activation of the β-catenin pathway [175]. CatE induces growth arrest and apoptosis in human prostate carcinoma tumor cell lines without affecting normal cells by catalyzing the proteolytic release of soluble TNF-related apoptosis-inducing ligand from the cell surface [176]. In addition, CatE was demonstrated to enhance the sensitivity of tumor cells to antitumor drugs [177].

The neutrophil peptidase CatG increases cell motility by proteolytic cleavage of cell surface proteins. It induces the E-cadherin-dependent aggregation of MCF-7 cells [178]. E-cadherin-based cell–cell junctions are regulated by CatG promotion of E-cadherin/catenin and E-cadherin/protein kinase D1 complex formation and Rap1 activation in MCF-7 cells [179]. CatG also activates protease-activated receptor 4 that triggers cell membrane blebbing, a mechanism recognized as an important regulator of cell migration, cancer cell invasion, and vesicular content release [180].

Tumor angiogenesis is another important mechanism during tumor progression. The hypoxic TME activates several signaling molecules, including VEGF, platelet-derived growth factor, interleukins (ILs), and TGF-β, which all promote the proliferation of endothelial cells. Proteolysis importantly contributes to angiogenesis, as it enables the migration and invasion of endothelial cells via ECM degradation, regulates the activity of cytokines and growth factors important for angiogenesis, and releases pro- and antiangiogenic factors [69,181].

In addition to the promotion of angiogenesis by degrading ECM [146], CatB enhances angiogenesis by degrading matrix-associated angiogenesis inhibitors, such as the endogenous tissue inhibitors of metalloproteases TIMP-1 and TIMP-2 [182]. Additionally, by degrading the ECM, CatB also releases growth factors bound to ECM proteins such as VEGF and TGF-β [75]. Next, CatL promotes invasion and integration of circulating endothelial progenitor cells into ischemic tissue that is required for the formation of new blood vessels [183] and that contributes to angiogenesis by releasing growth factors from the ECM (reviewed in [90]). In human gastric cancer, CatL also contributes to angiogenesis by regulating the CDP/Cux/VEGF-D pathway [84].

CatS generates the antiangiogenic peptides canstatin and arrestin by cleaving collagen type IV and proangiogenic γ2 fragments by cleaving laminin [184]. CatS has also been suggested to interact with VEGF during angiogenesis, [154]. In the establishment and functional development of tumor vasculature, important roles were also recognized for CatH [185] and CatK [70,146]. Pro-CatD and mature CatD also possess proangiogenic activity [114,186] and have been suggested to cleave and release proangiogenic basic fibroblast growth factor from the ECM [187] and to activate VEGF [188]. The proangiogenic role of CatD was further demonstrated by its activation of MAPK and PI3K/Akt signaling via a nonproteolytic mechanism present at higher nonacidic pH in the pre-TME [114,116]. Conversely, CatD is involved in the degradation of antiangiogenic factors, such as angiotatin, pro- lactin, and endostatin [70,114]. Furthermore, CatE inhibits angiogenesis by upregulating the antiangiogenic mediators IL-12 and endostatin [189]. Finally, CatG upregulation in cancer cells promotes tumor vascularization via upregulation of TGF-β signaling, VEGF, and monocyte chemotactic protein 1 [190].

The role of lysosomal peptidases in immune escape mechanisms in cancer

Eliminating cancer cells is the ultimate goal of the immune response during cancer immunosurveillance and immunotherapy. CTLs and NK cells are the key effectors in this process. CTL activation is an antigen-specific process requiring specific antigen recognition, activation, and differentiation into effector CTLs, whereas NK cells exist in a preactivated state and can rapidly and effectively kill tumor cells that have downregulated major histocompatibility complex class I molecules (reviewed in detail in [191]). Furthermore, whereas CTLs kill differentiated tumor cells, NK cells also have the ability to kill stem-like tumor cells [192,193]. Both CTLs and NK cells deploy the same killing mechanisms, through either the death receptor pathway or cytotoxic granule release [194]. Cytotoxic granules contain proforms of perforin and several peptidases, including granzymes (granzymes A, B, H, M,
and K in humans) [195]. Perforin is a calcium-dependent pore-forming protein that requires proteolytic removal of 20 amino acids at its C terminus for liberation of its C2 domain and activation. Perforin release and binding to the cell membrane is required for granzyme entry and apoptosis induction in target cells [196]. CatL has been implicated in the C-terminal processing and activation of perforin, as the selective inhibition of CatL reduced perforin activation and the killing capacity of human NK cell lines and primary mouse CTLs. However, in vivo, CatL deficiency reduced the amount of active perforin but did not affect the overall cytotoxicity of NK cells in mice [197]. Granzymes are serine peptidases that are stored in cytotoxic granules as inactive precursors that require the removal of the N-terminal dipeptide for their activation [198]. Even though CatC has an essential role in the in vivo activation of granzymes A and B, residual granzyme B activity is sufficient to combat viral infection in CatC−/− mice [199]. Furthermore, CatH has been identified as an additional progranzyme convertase [200].

The endogenous inhibitor cystatin F (CysF), a member of the type II cystatin family, predominantly acts on peptidases located within the endo/lysosomal system, including cytotoxic granules. The molecular form of CysF governs its inhibitory profile. After synthesis, CysF forms disulfide-linked dimers that do not inhibit the C1 family of cysteine peptidases but strongly inhibit legumain through a distant, second binding site [201]. N-terminal cleavage after CysF translocation to endo/lysosomes [202] produces active monomeric CysF that is a strong inhibitor of cathepsins C, H, and L [203,204]. Additionally, secreted CysF can be internalized, transported to endo/lysosomes, and, as such, can regulate cysteine peptidase activity in trans [49,205].

In NK cells, CysF was shown to reduce granule-mediated cytotoxicity by regulating the activity of the main granzyme convertases, cathepsins C and H [49]. Furthermore, increased CysF levels and decreased CatC and CatH levels are associated with target-induced inactivation of NK cytotoxicity, referred to as ‘split anergy’ [206]. Split anergy of NK cells can be triggered through interaction with tumor cells and monocytes and is characterized by high cytokine secretion and reduced efficacy in killing target cells [206]. Increased CysF levels were also detected in anergic CTLs [207]. Recently, CysF was also found in CD4+ T cells that acquired cytotoxic functions during long-term cultivation [208]. In contrast to most other type II cystatins, which are generally downregulated in tumors [62], CysF was found to be markedly upregulated in several types of cancer. In colorectal tumors, high CysF mRNA levels were shown to correlate with an increased risk of liver metastasis and poor survival [209,210]. Furthermore, CysF gene expression was shown to be higher in glioblastoma tissues than in normal brain tissues, and CysF mRNA levels were shown to correlate with shorter patient survival [211,212]. Finally, CysF was found to be expressed in patient-derived glioblastoma stem-like cells [211]. Recently, it was shown that extracellular CysF attenuates granzyme-mediated cytotoxicity in CTLs [213] and decreases the susceptibility of a glioblastoma cell line to NK cytotoxicity [214]. Apart from the effects on properforin and granzyme activation, increased extracellular CysF levels can affect the activity of immune cells through several additional mechanisms.

CatC−/− mice exhibit reduced expression of the β2 integrin receptors CD11c and CD11b on CTLs and CD11c on dendritic cells. These β2 integrin receptors are adhesion and signaling molecules that are critically important for cell-to-cell contact and leukocyte recruitment to inflammation sites [214]. Furthermore, apart from its role in activating perforin, CatL has been implicated in regulating the cytotoxic efficacy of CTLs by cleaving complement C3; namely, upon activation of T-cell receptor, CatL cleaves complement component C3 into C3a and C3b fragments, which in turn engage and activate their corresponding receptors (C3aR and CD46). Signaling through CD46 is necessary for optimal CTL cytotoxic activity [215]. Engagement of C3aR and CD46 is also important for the optimal survival and differentiation of CD4+ T lymphocytes toward the Th1 phenotype [216]. In CD4+ lymphocytes, CD46 costimulation also induces the expression of legumain, which processes single-chain CatL into its active two-chain form in human CD4+ T lymphocytes [217]. Inhibition of legumain activity in human CD4+ T lymphocytes reduces the generation of the CatL active forms and C3a and induction of IFN-γ-secreting cells by approximately 50% [217].

Conventional CD4+ lymphocytes cannot kill cancer cells directly; however, by secreting various cytokines, they play a significant role in shaping antitumor immune responses. A subset of Th17 helper lymphocytes plays an important role in cancer-related inflammation, which can be unfavorable or beneficial, depending on the setting and cancer type [218]. Both CatL and CatS have been implicated in the differentiation of the Th17 subset. CatL is an intrinsic promoter of Th17 development in CD4+ cells [219], and cell differentiation can be blocked by specific exogenous CatL inhibitors [220]. In mice, conventional CD4+ cells more readily differentiate to the Th17 cell type when lacking an endogenous CatL inhibitor, serpin B1 [220].
Through activation of the protease-activated receptor 2 receptor on dendritic cells, which drives IL-6 production and secretion, CatS has been implicated in the generation and expansion of Th17 lymphocytes [221].

Regulatory T cells are key factors in tumor immune escape, as they can inhibit the activation and differentiation of CD4+ helper T cells and CTLs to induce reactivity against tumor-expressed antigens through a variety of mechanisms [222]. It was shown that CatS inhibition enhances the immunosuppressive activity of regulatory T cells under normal conditions, whereas, in the presence of tumor cells, CatS inhibits regulatory T cells and stimulates antitumor immunity by promoting CTL proliferation and survival [223]. Similar observations have been made in CatK−/− mice, in which regulatory T cells were potent suppressors of effector T lymphocytes under normal conditions [224].

Important components of the TME are tumor-infiltrating immune cells of myeloid origin, which are actively involved in bidirectional interactions with tumor cells. Tumor-associated macrophages (TAMs) constitute the major leukocyte population in tumors [225]. TAMs are generally categorized into classically activated M1 macrophages (which typically exert antitumor functions) and alternatively activated M2 macrophages (which can inhibit T-cell-mediated antitumor immune responses, promote tumor angiogenesis, and lead to tumor progression) [226]. In addition to converting macrophages toward the tumor-promoting phenotype [226], the TME drives the expansion of a heterogenic immature population of myeloid-derived suppressor cells (MDSCs). MDSCs have a profound effect on the course of the antitumor immune response and on the effector functions of adaptive immune cells [227]. MDSCs and TAMs also support cancer progression by enhancing cancer cell stemness [228,229] and resistance to chemotherapy [230], releasing proangiogenic peptides necessary for angiogenesis [231], facilitating tumor invasation into the circulation, and contributing to tumor metastasis.

Increased proteolytic activity of cysteine cathepsins has also been associated with the tumor-promoting roles of MDSCs [163,232]. Recently, it was shown that during MDSC differentiation, the overall levels of cysteine cathepsins increase, with the most pronounced increase in the activities of CatL and CatX. Blocking their activity with small-molecule inhibitors (CLIK-148 and Z9 for CatL and CatX, respectively) in tumor and immune cell cocultures showed that CLIK-148 significantly increases CD8+ cytotoxicity [233]. Proteomic analysis of MDSCs from metastatic tumors revealed decreased neutrophilic granule protein compared with that of those from nonmetastatic counterparts.

Neutrophilic granule protein is structurally similar to type II cystatins and can inhibit CatB and consequently reduce tumor vascularization, growth, and metastasis [234].

In a mouse model of hereditary polyposis, increased Cat activity was detected in macrophages and MDSCs, which were infiltrating the lesions. MDSCs were shown to depend on CatB activity, since CatB−/− mice failed to accumulate MDSCs [235]. Furthermore, treatment with anti-TNF-α antibodies reduced MDSC density and decreased the detectable activity of polyprotein-specific CatB in the same mouse model [236]. This is in line with a previous study showing that CatB deficiency abrogated the trafficking of TNF-α-containing vesicles to the cell membrane in different types of monocytic cells [237].

A mouse SCID-hu model of bone metastasis revealed that stromal-derived CatK may be an important factor in the colonization and growth of tumors in the skeleton. CatK was suggested to interfere with macrophage-regulated inflammatory processes in bones [238]. A further study on a model of bone metastasis in CatK−/− mice showed critical involvement of bone marrow macrophage-derived CatK in bone tumor progression [239]. In this model, macrophage infiltration was reduced in the absence of CatK and correlated with lower inflammation levels [239]. Bone marrow macrophage-derived CatK was suggested to be involved in pathways that are driven by chemokine (C-C motif) ligand 2 and cyclooxygenase 2 and contribute to tumor progression and bone metastasis. Increased cyclooxygenase 2 activity, first associated with inflammation, is also frequently increased within the TME. This leads to increased synthesis of eicosanoid prostaglandin 2, which is a driver of the functional differentiation of TAMs and MDSCs [240,241]. Furthermore, it was shown that cathepsins are involved in post-translational cyclooxygenase 2 maturation and catalytic regulation, as their inhibition with the broad-spectrum Cat inhibitors E64d and ALLn was shown to block cyclooxygenase 2 maturation, resulting in diminished prostaglandin 2 formation [242]. Furthermore, CatK induced the overexpression of CatB, another important driver of tumor progression [239].

Macrophage-derived CatX was found to facilitate cancer cell invasion through the Arg-Gly-Asp (RGD) motif in its prodomain, which regulates interactions with integrins and the ECM [235]. Genetic ablation of CatS leads to the depletion of several proinflammatory chemokines, most notably the chemokine (C-C motif) ligand 2, which is required for the recruitment of MDSCs and TAMs. This regulation is transcriptionally mediated. CD74 (also
known as the major histocompatibility complex II chaperone invariant chain) is cleaved by CatS in endosomes, resulting in the release and nuclear translocation of its intracellular domain and the activation of transcription factor NF-κB, which transcriptionally regulates chemokine (C-C motif) ligand 2 expression [243].

Chemotherapy-induced MDSC depletion is generally favorable in tumor therapy; however, it was shown that cysteine cathepsins play an important role in some unfavorable off-target effects of chemotherapy. It was shown that 5-fluorouracil and gemcitabine, which selectively target and kill MDSCs, indirectly induce lysosomal membrane permeabilization and CatB leakage into the cytoplasm. Upon lysosomal membrane permeabilization, CatB was shown to directly interact with the leucine-rich repeat domain of NLRP3 and activate the inflammasome, the multiprotein platform for caspase-1 activation, which is necessary for conversion of pro-IL-1β into mature IL-1β. This leads to IL-1β secretion, which stimulates CD4⁺ T lymphocytes to produce IL-17, potentially leading to angiogenesis and subsequent tumor relapse [244]. Similarly, the commonly used chemotherapeutic paclitaxel was shown to increase TAM infiltration into the tumor site, which contributes to increased Cat activity within the TME. An in vitro study showed that macrophage-derived CatS and CatB, but not CatC and CatL, protect tumor cells against cell death induced by paclitaxel, etoposide, and doxorubicin [245].

Lysosomal peptidases in neurodegeneration

Neurodegeneration refers to the progressive loss of neuronal structure or function and can lead to devastating neurological conditions, such as Parkinson’s disease (PD), AD, and ALS. Impaired endo/lysosomal systems have been linked to the pathogenesis of neurodegenerative diseases and disrupted cellular homeostasis, thus contributing to neurodegeneration [246].

Lysosomal peptidases in brain pathologies related to misfolded proteins

Misfolded proteins that cause neurodegeneration are generated over the course of aging by post-translational modifications of native proteins or genetic mutations of otherwise nonpathogenic proteins [247]. In several neurodegenerative diseases, specific proteins begin to aggregate in individual brain regions at early, commonly nonsymptomatic stages of the disease, whereas additional brain regions become involved in the advanced stages of the disease [248]. Misfolding and aggregation of amyloid beta (Aβ) protein in senile plaques and tau protein in neurofibrillary tangles represent the most widely accepted pathogenic markers of AD [249,250]. However, another early feature of AD is lysosomal dysfunction, and accruing evidence suggests that lysosomal peptidases may be key pathogenic players (Table 2) [251,252].

Aspartyl peptidase CatD degrades both Aβ [253–255] and tau [256,257] and is strongly implicated in the pathogenesis of AD [258]. In AD patients, CatD levels are high in cortical and hippocampal neurons [259], amyloid plaques, and cerebrospinal fluid [260–262]. It has been suggested that CatD is also involved in the proteolysis of both lipid-free recombinant full-length human apolipoprotein E (apoE) and lipidated human plasma full-length apoE4 into toxic peptide, contributing to the progression of AD [263]. Additionally, another aspartyl peptidase, CatE, processes lipid-free recombinant human apoE to a much greater extent than lipidated apoE [263] and appears to be involved in neurodegeneration associated with brain ischemia and aging [264,265]. CatE is present in senile plaques in AD brains [266] and exhibits increased expression and lysosomal localization in cortical and brainstem neurons of aged rats [264].

Cysteine cathepsins are also associated with neurodegeneration (Table 2) [14,252]. Among them, CatB and CatL might be crucial in intracellular catabolism related to age-associated changes that lead to neuronal death [265,267]. High CatB and CatL levels were found in neurons and amyloid plaques in AD brain [268]. Conversely, mice lacking CatB and CatL exhibited atrophy in cerebral and cerebellar brain regions, suggesting the necessity of these cathepsins for neuronal development [269]. Furthermore, suppression of CatB and CatL by exposing cultured hippocampal slices to a selective Cat inhibitor provoked changes similar to those occurring during brain aging, for example, an increased number of lysosomes and the formation of neurites [270]. Nevertheless, the cysteine cathepsins B, L, and S were identified as enzymes possessing β-secretase activity for the cleavage of amyloid precursor protein (APP) into toxic Aβ peptide [271]. Among them, CatB in secretory vesicles is most strongly defined as a β-secretase for the production of the neurotoxic Aβ peptide in AD [272–274].

CatB shows a clear preference for cleaving wild-type β-secretase substrate, whereas it shows essentially no activity for Swedish mutant β-secretase substrate [271,274]. Inhibition by the cysteine peptidase inhibitor E64d and related inhibitor CA-074Me (which preferentially inhibits intracellular CatB) reduces brain Aβ peptide levels and improves memory in an AD mouse.
| Type of Cat | Function                                                                 | Pathogenesis       | References |
|------------|--------------------------------------------------------------------------|--------------------|------------|
| CatD       | Proteolytic cleavage of Aβ and tau protein                               | AD                 | [253–257]  |
|            | Proteolysis of apoE into toxic peptide                                  | AD                 | [263]      |
|            | Proteolysis of α-syn; disturbance in CatD function leading to pathogenesis| PD                 | [303,305,306]|
|            | Involved in 6-OHDA-induced apoptosis of dopaminergic cells               | PD                 | [312]      |
|            | Mutations in CatD gene                                                  | NCL type 10        | [363]      |
| CatE       | Proteolysis of apoE into toxic peptide                                  | Aging, AD          | [263]      |
| CatB       | β-secretase activity in APP cleavage into toxic Aβ peptide; preference for cleaving wild-type β-secretase substrate | AD                 | [271–274]  |
|            | Proteolysis of α-syn; formation of intracellular α-syn aggregates        | PD                 | [306,309]  |
|            | Involved in motor neuron degeneration                                    | ALS                | [317]      |
|            | Proteolytic degradation of mitochondrial transcription factor A          | Neuroinflammation, aging | [331] |
|            | Involved in caspase-1 activation leading to secretion of interleukin-1β; involved in caspase-11 activation |              | [343,344]  |
|            | Loss of CatB activity leads to accumulation of free cholesterol in late endosomes | NPC                | [371,372]  |
| CatL       | β-secretase activity in APP cleavage into toxic Aβ peptide              | AD                 | [271]      |
|            | Proteolysis of α-syn                                                    | PD                 | [306]      |
|            | Involved in 6-OHDA-induced apoptosis of dopaminergic cells               | PD                 | [313]      |
|            | Contributes to inflammatory responses when released from activated microglia | Neuroinflammation  | [337]      |
|            | Loss of CatL activity leads to accumulation of free cholesterol in late endosomes | NPC                | [372]      |
| CatS       | β-secretase activity in APP cleavage into toxic Aβ peptide              | AD                 | [271]      |
|            | Degrades monomers and dimers of the Aβ peptide and APP in vitro          | AD                 | [289]      |
| CatC       | Promotes M1 microglia polarization via the Cat2-dependent PKC/p38MAPK/NF-κB pathway | Neuroinflammation  | [345]      |
| CatF       | Mutations in CatF gene                                                  | NCL type 13        | [365–369]  |
|            | Accumulation of eosinophilic granules and lipofuscin in neurons is increased in association with decreased CatF expression | NCL type 13        | [364]      |
| CatX       | Proteolytic cleavage of the C-terminal end of γ-enolase, abolishing its neurotrophic activity | Aging, AD          | [294–296]  |
|            | Involved in 6-OHDA-induced apoptosis of dopaminergic cells               | PD                 | [314,315]  |
|            | Contributes to inflammatory responses when released from activated microglia | Neuroinflammation  | [349,352]  |
| Legumain   | Phosphorylation of tau protein; degradation of tau protein               | Aging, AD          | [298,299]  |

Model. Conversely, E64d has no effect in this model expressing the Swedish mutant β-secretase site of APP [271,274–277]. Kindy et al. showed improved memory deficits after CatB gene knockout in an AD mouse model expressing the wild-type β-secretase site of APP that is present in most AD patients [278]. Conversely, CatB degraded Aβ via C-terminal truncation, leaving its role in Aβ metabolism unclear [279,280]. Mueller-Steiner et al. demonstrated that CatB actually reduces Aβ peptide levels, especially the aggregation-prone species Aβ1–42, through proteolytic cleavage. They suggested that inhibition or loss of CatB activity could interfere with the protective function of CatB and thus promote the development of AD [280]. A recent study by Oberstein et al. on cultured astrocytes showed varying roles of CatB in Aβ regulation that might depend on different cellular localizations of active CatB. Nonlysosomal CatB mediated Aβ production in astrocytes, while Aβ degradation depended on lysosomal CatB and the production of Aβ peptides; this highlights the importance of considering organelle targeting in drug development to promote Aβ degradation [281]. Nevertheless, elevated CatB levels have been detected in AD patient brains in membrane-bound organelles, degenerating neuronal perikarya, reactive astrocytes, and extracellularly near neuritic plaques [268,282,283]. In addition, CatB plasma levels are elevated in AD patients [284,285]. Therefore, CatB has been recognized as a crucial pathogenic factor and potential target in AD [286].

Another enzyme with β-secretase activity that is associated with the pathogenesis of AD is CatS [271]. Transfection of human kidney cells with CatS increased Aβ secretion, whereas the Cat inhibitor E64d reduced this secretion [287]. CatS is weakly detected in normal human brain, whereas CatS immunoreactivity is increased in AD brain regions rich in neuritic plaques, supporting its involvement in AD pathology [257].

Table 2. Significance of lysosomal peptidases in neurodegeneration.
was observed in tangle-bearing neurons, astrocytes, and rare senile plaques in AD brain [288]. In addition, Liuzzo et al. demonstrated that CatS can degrade Aβ peptide monomers and dimers in vitro [289]. It is known that Aβ peptides are taken up predominantly by microglia and are accumulated and degraded in microglial endo/lysosomal systems [290]. Thus, microglial CatS may assist in the extracellular clearance of intracellularly formed Aβ or soluble Aβ and modulate Aβ peptide levels at the very initial stages of peptide aggregation, which in turn may affect Aβ neurotoxicity [291]. Besides CatS, enhanced CatL and CatH levels were found in the majority of astroglia and microglia in the hippocampus of AD patients, both within and outside senile plaques [292,293], indicating the pathogenic role of CatL and CatH in age-related neurodegeneration.

Another lysosomal cysteine peptidase strongly linked to age-related neurodegeneration is CatX. High levels and proteolytic activity of CatX have been observed in degenerating brain regions of transgenic AD mouse models and around senile plaques in AD patient brains [294,295]. A transgenic AD mouse model revealed CatX upregulation in microglial cells surrounding amyloid plaques and CatX colocalization with its target γ-enolase in the vicinity of the plaques [294,295]. Furthermore, CatX contributes to Aβ-related neurodegeneration through proteolytic cleavage of the C-terminal dipeptide of γ-enolase, abolishing its neurotrophic and neuroprotective activity [295]. Consequently, γ-enolase cannot impair Aβ-induced apoptosis through neurotrophin receptor p75NTR signaling [296]. Furthermore, a comprehensive comparative gene expression analysis of mouse models of AD, multiple sclerosis, and stroke found that CatX is one of the eighteen genes whose expression is increased in all three models of central nervous system (CNS) disorders [297].

In addition, legumain, which is activated in aging and AD brains [298], is involved in tau phosphorylation by inactivating protein phosphatase 2 inhibitor I2 [299]. Legumain is also involved in tau degradation, thereby abolishing its microtubule assembly function and inducing its aggregation that leads to neurodegeneration [298].

The accumulation of misfolded proteins plays a central role in the pathogenesis of PD and impairs lysosomal function [300]. The crucial pathological event in PD involves the aggregation of alpha-synuclein (α-syn) from intermediate soluble oligomers to structurally complex and insoluble fibrils found in Lewy bodies and neurites [301]. The lysosomal degradation pathway is mostly responsible for the clearance of α-syn oligomers, and disturbance in lysosomal function has been linked to the accumulation of α-syn oligomers and α-syn-mediated cell death [302].

CatD was the first lysosomal peptidase found to protect against α-syn aggregation and toxicity in mouse models [303–305]. In vitro and in vivo studies demonstrated that CatD mediates the lysosomal proteolysis of α-syn under physiological conditions [303,305,306]. In agreement, overexpressed CatD was found to effectively degrade α-syn in dopaminergic cells, whereas CatD-deficient mice accumulated insoluble α-syn in the brain, thereby facilitating α-syn toxicity [303]. Similar results, that is, α-syn accumulation in CatD-deficient animals and neuroprotection against α-syn toxicity in CatD-overexpressing neuroglioma cells, were also observed by Qiao et al. [304]. Moreover, using CatD-deficient lysosomes, CatD has been demonstrated to be the main lysosomal enzyme involved in α-syn degradation [305]. Recently, damging variants of CatD were found to be genetically linked to lysosomal dysfunction and PD pathology in a large screening of PD patients [307]. Furthermore, an additional study showed that a PD-associated CatD variant (A239V) exhibited increased enzymatic activity accompanied by increased α-syn levels [308].

Conversely, cysteine cathepsins have been shown to be essential in lysosomal degradation of α-syn. Using lysosomal extracts and mass spectrometry analysis, CatD was found to only generate C-terminal α-syn fragments, whereas the majority of α-syn degradation was associated with CatL, and to a lesser extent with CatB [306]. In a cell-based study using the CatB inhibitor CA-074Me and CatD inhibitor pepstatin, CatB, but not CatD, was found to be the major enzyme involved in fibril-induced formation of intracellular α-syn aggregates. Similar results were obtained using CatB knockdown [309]. Further studies are therefore needed to resolve this discrepancy.

**Lysosomal peptidases in progressive degeneration accompanied by neuronal loss**

Another feature of PD is a progressive degeneration of the dopaminergic projection in the substantia nigra compacta (SNc), which results in loss of dopaminergic neurons in the SNc. The important role of certain cysteine cathepsins in neurodegenerative disorders is becoming well established in acute pathological conditions and chronic diseases with inflammatory pathologies such as PD (Table 2; reviewed in [65]). [310,311]. The lysosomal proteolytic system participates in the apoptosis of neuronal-like cells induced by 6-hydroxydopamine (6-OHDA), a common neurotoxin model of PD [312].
Increased CatB and CatD expression has been shown in a 6-OHDA model of PD. Cells treated with pepstatin A, a CatD inhibitor, showed a significant decrease in cell death; however, CA-074Me, a CatB inhibitor, failed to protect cells from 6-OHDA-induced cell death [312]. Also, other cysteine peptidases, for example, CatL and CatX, play a role in the apoptosis of dopaminergic neurons. CatL mediates 6-OHDA-induced apoptotic events leading to PD-related neurodegeneration [313]. As such, reports have shown increased CatL expression in dopamine neurons in ipsilateral SNc of a rat PD model and in PD patients [310]. An in vitro study revealed that also CatX promotes 6-OHDA-induced apoptosis and subsequent neuronal toxicity, and CatX inhibition exerts potent neuroprotection of dopaminergic-like neuronal cells, designating peptidases as pathogenic factors in the progressive loss of dopaminergic neurons [314]. Indeed, another in vivo study revealed CatX upregulation in a 6-OHDA model of PD. 6-OHDA injection into the medial forebrain bundle increased CatX expression and activity in the SNc at the ipsilateral side, with the simultaneous reduction in numerous dopaminergic nigrostriatal neurons. This prominent CatX upregulation was restricted to dopaminergic neuronal cells at early time points after the injection, whereas at late time points, CatX upregulation was restricted to glial cells concentrated in the ipsilateral SNc [315].

Another neurodegenerative disease where progressive neuronal loss is present is ALS. ALS is characterized by selective degeneration and death of motor neurons associated with the accumulation of misfolded proteins and insoluble inclusions [316]. At first, only CatB was found to be involved in motor neuron degeneration, whereas cathepsins H, L, and D were not significantly affected in ALS patients [317]. However, further studies showed that the expression of CatB, CatL, and particularly CatD increases in ALS spinal cord with a concomitant change in the distribution and lysosomal associations of CatD [318]. ALS model mice revealed that the expression and protein levels of cathepsins B, L, S, X, and D all increased in the spinal cord in ALS mice, generated by mutating the copper/zinc superoxide dismutase (SOD1) gene [294,318,319]. Additionally, a cDNA microarray analysis on postmortem spinal cord specimens of four sporadic ALS patients revealed major changes in mRNA expression of 60 genes, including increases in CatB and CatD [320]. Nevertheless, CatB-knockout mice showed a lower rate of motor neuron death after nerve injury [321], suggesting that CatB inhibition is beneficial for motor neuron survival. It is therefore likely that lysosomal enzymes, such as cathepsins, are activated in the ALS spinal cord and may contribute to the disease [318].

Microglial lysosomal peptidases promote neuroinflammation

Accumulating evidence suggests that chronic innate neuroinflammation mediated by microglia and astrocytes is involved in the progressive nature of neurodegenerative disorders [322]. During neuroinflammation, activated microglia and astrocytes release a variety of cytokines, chemokines, and toxic factors, which may lead to subsequent neuronal toxicity. This is accompanied by oxidative stress [323], mitochondrial dysfunction [324], and activation of the apoptotic cascade [325,326], all of which lead to aggressive neuronal loss and exacerbate neurodegeneration [327–330]. In addition to inflammatory molecules, activated microglia also secrete lysosomal peptidases, which support various immune functions [290,331,332]. Inflammatory stimuli such as lipopolysaccharide (LPS), which also induces death of nigral dopaminergic neurons through microglial activation, substantially increase microglial secretion of lysosomal peptidases [289,333–336]. In the microglia cell line BV2, LPS exposure leads to increased levels of the cysteine cathepsins B, K, S, and X in culture supernatants [336]. Substantially increased CatL secretion from microglia has been observed in response to LPS treatment for 1 h, which is earlier than the upregulation of proinflammatory cytokines, indicating that the earlier release of lysosomal CatL in microglia may contribute to inflammatory responses [337]. In addition, CatL inhibition alleviates the microglia-mediated neuroinflammatory responses through caspase-8 and NF-κB pathways [338]. Furthermore, cathepsins B [339], L [338], H [340], C [341], and X [342] are upregulated in different brain regions following LPS-induced neuroinflammation.

Microglial CatB has been extensively studied in neuroinflammation. Cytosplasmic CatB enhances the activation of caspase-1, therefore promoting the microglial production and secretion of proinflammatory cytokine IL-1β [343] through the pyrin domain-containing protein 3 inflammasome-independent processing of pro-caspase-3 in phagolysosomes [344]. The leakage of CatB from the endo/lysosomal system during aging is associated with the proteolytic degradation of mitochondrial transcription factor A, which can stabilize mitochondrial DNA. Therefore, microglial CatB could function as a major driver of inflammatory brain diseases and brain aging (reviewed in [331]). Similarly, the expression of microglia-secreted CatC is enhanced
during CNS inflammation. CatC expression in the brain is induced predominantly in activated microglia [341], and CatC plays a role in promoting chemokine production in CNS inflammation [345]. CatC promotes microglia M1 polarization and aggravates neuroinflammation via the Ca2⁺-dependent PKC/p38MAPK/NF-κB pathway [346]. Similarly, the expression of microglia-secreted CatS is increased during CNS inflammation and aging in mice [319]. Altered CatS expression is controlled by a built-in molecular clock in cortical microglia; the circadian expression of CatS is involved in diurnal variations of synaptic strength via proteolytic modification. CatS has also been associated with some sleeping disorders, as its genetic ablation reduces synaptic strength during sleep by inducing hyperlocomotor activity that is required to obtain novel information after waking [347].

CatX has also been associated with inflammatory processes leading to neurodegeneration. It is disproportionately expressed and secreted by microglia and astrocytes in response to neuronal damage and inflammatory stimulus, both in vitro and in vivo [336,348–350]. In vitro, the inflammatory stimulus LPS substantially increases CatX secretion from microglia, leading to neurodegeneration mediated by microglia activation [336,349]. This was confirmed by the CatX-specific inhibitor AMS36, which suppressed the production of proinflammatory molecules and attenuated cytokine release from activated microglial cells, leading to reduced microglia-mediated neurotoxicity [349]. In vivo, unilateral LPS injection into the striatum increased CatX expression and activity in the striatum and surrounding areas on the ipsilateral side. This prominent CatX upregulation was restricted to activated microglia and reactive astrocytes (Fig. 1B). Moreover, administration of a CatX inhibitor along with LPS injection revealed the potentially protective role of such inhibitors in neuroinflammation-induced striatal lesions [342]. Additionally, dendritic cells in the aging brains of mice have increased CatX protein levels, indicating its role in neuroinflammation [351]. Allan et al. showed that CatX-deficient mice have reduced neuroinflammation and decreased circulating IL-1β levels during experimental autoimmune encephalomyelitis, a well-known model of multiple sclerosis [352]. Multiple sclerosis is an autoimmune disease characterized by immune-mediated inflammation, which attacks the myelin sheath. Hypomethylation of the CatX locus has been proposed as an epigenetic risk factor for multiple sclerosis [353].

Several observations suggest that also other cysteine cathepsins play a role in immune-mediated inflammation involved in multiple sclerosis. Markedly increased levels of CatB and CatS in peripheral blood mononuclear cells, serum, and cerebrospinal fluid of multiple sclerosis patients have been determined [354–357] and confirmed in an experimental models of autoimmune encephalomyelitis [358]. Predominant autoantigens, for example, myelin basic protein and myelin oligodendrocyte glycoprotein, are targets for CatS processing in antigen-presenting cells [358,359]. Furthermore, altered CatS expression has been linked with disease activity [354]. Finally, a recent study showed that altered expression of cysteine cathepsins mitigates fast endo/lysosomal degradation of the immunodominant epitope 40–48 of myelin oligodendrocyte glycoprotein [360].

**Lysosomal peptidases in brain pathologies related to lysosomal storage disease**

Mutations in genes encoding proteins involved in lysosomal function cause lysosomal storage diseases, which are characterized by the progressive accumulation of undegraded substrates inside endo/lysosomal compartments [361,362]. In the CNS, neuronal ceroid lipofuscinoses (NCLs) are known to be caused by inactivation mutations in Cat genes (Table 2), namely defects in CatD and Cat F (CatF), which result in type 10 and type 13 of NCL, respectively [362]. In particular, NCL10 is caused by mutations in the CatD gene due to autosomal recessive inheritance [363], accompanied by congenital, late infantile, or juvenile onset. To date, 21 mutations have been identified that affect the CatD gene, whereas only nine mutations have been confirmed to be pathogenic and linked to the development of NCL10 (reviewed in [362]). A study on CatF-deficient mice revealed that CatF is also involved in NCL-like neurodegenerative disorders, as CatF-deficient mice developed progressive neurological features with onsets at 12–16 months and died prematurely. Additionally, CatF-deficient mice accumulated large amounts of autofluorescent lipofuscin in the CNS, which is a characteristic of NCLs [364]. Further studies confirmed that mutations in the CatF gene result in NCL type 13, an adult-onset form of NCL, also known as type B Kufs disease [365–369]. To date, nine mutations with recessive inheritance were associated with NCL13, and multiple lines of evidence suggest that CatF variants are indeed pathogenic mutations (reviewed in [362]).

Nevertheless, no human patient with dysfunctional CatB and CatL was identified so far. Like CatD-deficient mice [370], CatB- and CatL-deficient mice also display pronounced lysosomal storage diseases that lead to extensive neuronal death in the CNS and to the development of pronounced brain atrophy due
to massive apoptosis of neurons in the cerebral cortex and cerebellar Purkinje and granule cell layers. However, prior to neuronal cell death, CatB- and CatL-deficient neurons develop a lysosomal storage disease similar to human NCL, suggesting that CatB and CatL are essential for the maturation and integrity of the postnatal CNS [269,370]. CatB and CatL can compensate for each other in vivo, since only CatB<sup>−/−</sup>L<sup>−/−</sup> double-mutant mice develop neurodegeneration accompanied by pronounced reactive astrocytosis [269]. Nevertheless, cathepsins have been linked to another progressive lysosomal storage disease, Niemann-Pick disease type C (NPC), characterized by intracellular accumulation and redistribution of cholesterol in a number of tissues, including the brain [371]. The increased levels and activities and altered subcellular distribution of CatB and CatD in the cerebellum of mouse brain with NPC pathology have been associated with the underlying cause of neuronal vulnerability in NPC brains. However, a study by Cermak et al. showed that CatB and CatL, but not CatD, represent major lysosomal peptidases that control lysosomal function. The inhibition of CatB and CatL, but not CatD, leads to lysosomal impairment. Furthermore, loss of CatB and CatL activity leads to the accumulation of free cholesterol in late endo/lysosomes, resembling a phenotype characteristic of Niemann-Pick disease type C [372].

**Conclusions**

Lysosomal peptidases represent a pool of enzymes involved in both intracellular catabolism of waste proteins and important physiological functions, such as apoptosis, processing hormones, activating other enzymes, and maintaining homeostasis of immune and neuronal cells. If lysosomal peptidase activity is not properly controlled, excessive protein degradation may lead to severe cell and tissue damage or changes associated with numerous pathologies, the most investigated being cancer, neurodegeneration, and immune disorders. As tumors progress from transformed cells toward highly malignant cells, they pass through several stages that require the action of peptidases. They induce EMT to the malignant cell phenotype and the escape of cancer cells from the primary site, breaking down connective barriers of the ECM and basement membrane during cell migration and extravasation at distant sites during metastases. Lysosomal peptidases are also involved in mechanisms preventing tumor cell apoptosis and immune surveillance. Conversely, they may promote the antitumor action of cytotoxic immune cells, such as CTLs and NK cells. Lysosomal peptidase dysfunction is also typical for neurodegenerative diseases. It can result in compromised proteolytic degradation of misfolded proteins, formation of amyloid aggregates, neuronal loss, and neuroinflammation. Endogenous protein inhibitors of lysosomal peptidases may counterbalance the harmful proteolytic action during pathological processes; however, they may also affect the processes leading to disease regression, such as antitumor immune responses, tumor cell apoptosis, or dissolving of protein aggregates. The regulation of lysosomal peptidases as a therapeutic approach must be fine-tuned either by specific peptidase inhibitors or by transcription/translation editing and must focus on the harmful fractions of particular peptidases by using advanced delivery systems.

**Acknowledgements**

This work was supported by the Slovenian Research Agency (grant numbers P4-0127, J4-1776 to JK; J3-3071 to AM; J3-2516 to MPN; and J3-9267 to AP). We thank Dr. Eva Lasic for critically reviewing a draft of this manuscript.

**Conflicts of interest**

There are no conflicts of interest to declare.

**Author contributions**

JK and AP designed the concept of the review manuscript. JK, AM, MPN, and AP prepared the draft manuscript. AP and AM prepared Fig. 1. AM prepared Table 1 and designed the graphical abstract. AP prepared Table 2. JK reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

**Data accessibility**

All original data are available from the corresponding author on request.

**References**

1 de Duve C. The lysosome turns fifty. *Nat Cell Biol*. 2005;7:847–9.
2 Underwood E. When the brain’s waste disposal system fails. *Knowable Mag*. 2018. http://dx.doi.org/10.1146/knowable-121118-1
3 Kuehnel W. *Color atlas of cytology, histology and microscopic anatomy*. Leipzig: Thieme Flexibook; 2003. http://www.amazon.com/Cytology-Histology-Microscopic-Anatomy-Flexibook/dp/1588901750
4 Conner SD, Schmid SL. Regulated portals of entry into the cell. *Nature*. 2003;422:37–44.
5 Doherty GJ, McMahon HT. Mechanisms of endocytosis. *Annu Rev Biochem*. 2009;78:857–902.
6 Luzio JP, Pryor PR, Bright NA. Lysosomes: fusion and function. *Nat Rev Mol Cell Biol*. 2007;8:622–32.
7 Kiss AL. Caveolae and the regulation of endocytosis. *Adv Exp Med Biol*. 2012;729:14–28.
8 Donaldson DG, Porat-Shliom N, Cohen LA. Clathrin-independent endocytosis: a unique platform for cell signaling and PM remodeling. *Cell Signal*. 2009;21:1–6.
9 Engqvist-Goldstein AEY, Drubin DG. Actin assembly and endocytosis: from yeast to mammals. *Annu Rev Cell Dev Biol*. 2003;19:287–332.
10 Rink J, Glhigo E, Kalaidzidis Y, Zerial M. Rab conversion as a mechanism of progression from early to late endosomes. *Cell*. 2005;122:735–49.
11 Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature*. 2011;469:323–35.
12 Castro-Obregon S. The discovery of lysosomes and autophagy. *Nat Educ*. 2010;3:49.
13 Česen MH, Pegan K, Spes A, Turk B. Lysosomal pathways to cell death and their therapeutic applications. *Exp Cell Res*. 2012;318:1245–51.
14 Repnik U, Stoka V, Turk V, Turk B. Lysosomes and lysosomal cathepsins in cell death. *Biochim Biophys Acta*. 2012;1824:22–33.
15 Rawlings ND, Barrett AJ, Thomas PD, Huang X, Bateman A, Finn RD. The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. *Nucleic Acids Res*. 2018;46:D624–32.
16 Zhang W, Lin Y. The mechanism of asparagine endopeptidase in the progression of malignant tumors: a review. *Cells*. 2021;10:1153.
17 Patel S, Homaei A, El-Seedi HR, Akhtar N. Cathepsins: proteases that are vital for survival but can also be fatal. *Biomed Pharmacother*. 2018;105:526–32.
18 van der Spoel A. Transport of human lysosomal neuraminidase to mature lysosomes requires protective protein/cathepsin A. *EMBO J*. 1998;17:1588–97.
19 Grisolano JL, Sclar GM, Ley TJ. Early myeloid cell-specific expression of the human cathepsin G gene in transgenic mice. *Proc Natl Acad Sci USA*. 1994;91:8989–93.
20 Burster T, Macmillan H, Hou T, Boehm BO, Mellins ED. Cathepsin G: roles in antigen presentation and beyond. *Mol Immunol*. 2010;47:658–65.
21 Zamolodchikova TS, Tolpygo SM, Svirshchevskaia EV. Cathepsin G—not only inflammation: the immune protease can regulate normal physiological processes. *Front Immunol*. 2020;11:411.
22 Fu Z, Akula S, Thorpe M, Hellman L. Potent and broad but not unselective cleavage of cytokines and chemokines by human neutrophil elastase and proteinase 3. *Int J Mol Sci*. 2020;21:651.
23 Burster T. Processing and regulation mechanisms within antigen presenting cells: a possibility for therapeutic modulation. *Curr Pharm Des*. 2012;18:1029–42.
24 Burster T, Knippschild U, Molnár F, Zhanapiya A. Cathepsin G and its dichotomous role in modulating levels of MHC class I molecules. *Arch Immunol Ther Exp (Warsz)*. 2020;68:25.
25 Pontious C, Kaul S, Hong M, Hart PA, Krishna SG, Lara LF, et al. Cathepsin E expression and activity: role in the detection and treatment of pancreatic cancer. *Pancreatology*. 2019;19:951–6.
26 Chain BM, Free P, Medd P, Swetman C, Tabor AB, Terrazzini N. The expression and function of cathepsin E in dendritic cells. *J Immunol*. 2005;174:1791–800.
27 Arnold D, Keilholz W, Schild H, Dumreis T, Stevanovic S, Rammensee H-G. Substrate specificity of cathepsins D and E determined by N-terminal and C-terminal sequence of a new clan of peptide pools. *Eur J Biochem*. 1997;249:171–9.
28 Noshioku T, Hashimoto K, Yamashita K, Liou S-Y, Kagamiishi Y, Maegawa H, et al. Involvement of cathepsin E in exogenous antigen processing in primary cultured murine microglia. *J Biol Chem*. 2002;277:4816–22.
29 Kageyama T, Takahashi K. A cathepsin D-like acid proteinase from human gastric mucosa: purification and characterization I. *J Biochem*. 1980;87:725–35.
30 Oberle C, Huai J, Reinecheck T, Tacke M, Rassner M, Ekert PG, et al. Lysosomal membrane permeabilization and cathepsin release is a Bax/Bak-dependent, amplifying event of apoptosis in fibroblasts and monocytes. *Cell Death Differ*. 2010;17:1167–78.
31 Mijanovic O, Petushkova AI, Brankovic A, Turk B, Solovieva AB, Nikitkina AI, et al. Cathepsin D—managing the delicate balance. *Pharmaceutics*. 2021;13:837.
32 Bischof J, Westhoff M-A, Wagner JE, Halatsch M-E, Trentmann S, Knippschild U, et al. Cancer stem cells: the potential role of autophagy, proteolysis, and cathepsins in glioblastoma stem cells. *Tumour Biol*. 2017;39:1010428317692227.
33 Höllén L, ParigiMA, Reinheckel T. Tumor cell- and microenvironment-specific roles of cysteine cathepsins in mouse models of human cancers. *Biochim Biophys Acta Proteins Proteom*. 2020;1868:140423.
34 Zhang X, Luo S, Wang M, Shi G-P. Cysteine and cathepsins in cardiovascular diseases. *Biochim Biophys Acta Proteins Proteom*. 2020;1868:140360.
35 Chen J-M, Rawlings ND, Stevens RAE, Barrett AJ. Identification of the active site of legumain links it to caspase-3, cispain and ginsapain in a new clan of cysteine endopeptidases. *FEBS Lett*. 1998;441:361–5.
Manoury B, Hewitt EW, Morrice N, Dando PM, Barrett AJ, Watts C. An asparaginyl endopeptidase processes a microbial antigen for class II MHC presentation. *Nature*. 1998;396:695–9.

Sepulveda FE, Maschulski S, Colisson R, Heslop L, Ghirelli C, Sakka E, et al. Critical role for asparagine endopeptidase in endocytic toll-like receptor signaling in dendritic cells. *Immunity*. 2009;31:737–48.

Stathopoulos C, Gangapala A, Mallett G, Flomerfelt FA, Liniary LP, Knight D, et al. PDL-1 inhibitory receptor downregulates asparaginyl endopeptidase and maintains Foxp3 transcription factor stability in induced regulatory T cells. *Immunity*. 2018;49:247–63.e7.

Mai C-W, Chung FF-L, Leong C-O. Targeting legumain as a novel therapeutic strategy in cancers. *Curr Drug Targets*. 2017;18:1259–68.

Boland B, Yu WH, Corti O, Mollereau B, Henriques MJ, et al. The refined 2.15 Å X-ray crystal structure of human liver cathepsin B: the structural basis for its dipeptidyl carboxypeptidase activities. *J Biol Chem*. 2010;285:13783–8.

Turk D, Guncar G, Podobnik M, Pungercar J, Strukelj B, Turk V. Crystal structure of porcine cathepsin H chain C-terminal carboxyl group defines cathepsin H aminopeptidase function. *Structure*. 1998;6:51–61.

Illy C, Quraishi O, Wang J, Purisima E, Vernet T, Mort JS. Role of the occluding loop in cathepsin B activity. *J Biol Chem*. 1997;272:1197–202.

Vasiljeva O, Dolinar M, Turk V, Turk B. Recombinant human cathepsin H lacking the mini chain is an endopeptidase. *Biochemistry*. 2003;42:13522–8.

Perišić Nanut M, Sabotić J, Švajger U, Jewett A, Kos J. Cystatin F affects natural killer cell cytotoxicity. *Front Immunol*. 2017;8:1459.

Turk B, Turk D, Turk V. Protease signalling: the cutting edge. *EMBO J*. 2012;31:1630–43.

López-Otín C, Bond JS. Proteases: multifunctional enzymes in life and disease. *J Biol Chem*. 2008;283:30433–7.

Neurath H. Proteolytic enzymes, past and future. *Proc Natl Acad Sci USA*. 1999;96:10962–3.

Soond SM, Kozhevnikova MV, Frolova AS, Savvateeva LV, Plotnikov EY, Townsend PA, et al. Lost or forgotten: the nuclear cathepsin protein isoforms in cancer. *Cancer Lett*. 2019;462:43–50.

Petushkova AI, Zamyatnin AA. Redox-mediated post-translational modifications of proteolytic enzymes and their role in protease functioning. *Biomolecules*. 2020;10:650.

Novicec M, Lenarčič B, Turk B. Cysteine cathepsin activity regulation by glycosaminoglycans. *Biomed Res Int*. 2014;2014:1–9.

Kos J, Mitrović A, Mirković B. The current stage of cathepsin B inhibitors as potential anticancer agents. *Future Med Chem*. 2014;6:1355–71.

Edginton-Mitchell LE, Bogoy M, Verdoes M. Live cell imaging and profiling of cysteine cathepsin activity using a quenched activity-based probe. In: Overkleeft HS, Florea BI, editors. *Activity-based proteomics: methods and protocols*. New York, NY: Springer Science+Business Media New York; 2017:45–59.

Edginton LE, Verdoes M, Bogoy M. Functional imaging of proteases: recent advances in the design and application of substrate-based and activity-based probes. *Curr Opin Chem Biol*. 2011;15:798–805.

Deu E, Verdoes M, Bogoy M. New approaches for dissecting protease functions to improve probe development and drug discovery. *Nat Struct Mol Biol*. 2012;19:9–16.

Verdoes M, Verhelst SHL. Detection of protease activity in cells and animals. *Biochim Biophys Acta Proteins Proteom*. 2016;1864:130–42.

Anes E, Azevedo-Pereira JM, Pires D. Cathepsins and their endogenous inhibitors in host defense during mycobacterium tuberculosis and HIV infection. *Front Immunol*. 2021;12:726984.

Breznik B, Mitrović A, T Lah T, Kos J. Cystatins in cancer progression: more than just cathepsin inhibitors. *Biochimie*. 2019;166:233–50.

Brix K, Dunkhorst A, Mayer K, Jordans S. Cysteine cathepsins: cellular roadmap to different functions. *Biochimie*. 2008;90:194–207.

Kos J, Lah TT. Cysteine proteinases and their endogenous inhibitors: target proteins for prognosis, diagnosis and therapy in cancer (review). *Oncol Rep*. 1998;5:1349–61.

Pišlar A, Kos J. Cysteine cathepsins in neurological disorders. *Mol Neurobiol*. 2014;49:1017–30.
66 Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
67 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
68 Pišlar A, Perišić Nanut M, Kos J. Lysoosomal cysteine peptidases - molecules signaling tumor cell death and survival. Semin Cancer Biol. 2015;35:168–79.
69 Olson OC, Joyce JA. Cysteine cathepsin proteases: regulators of cancer progression and therapeutic response. Nat Rev Cancer. 2015;15:712–29.
70 Khakset TP, Kwon TK, Kang SC. Cathepsins: potent regulators in carcinogenesis. Pharmacol Ther. 2019;198:1–19.
71 Navab R, Chevet E, Authier F, Di Guglielmo GM, Bergeron JJM, Brodi P. Inhibition of endosomal insulin-like growth factor-I processing by cysteine proteinase inhibitors blocks receptor-mediated functions. J Biol Chem. 2001;276:13644-9.
72 Massagué J. TGFbeta in cancer. Cell. 2008;134:215–30.
73 Yin M, Soikkeli J, Jahkola T, Virolainen S, Saksela O, Hölttä E. TGF-β signaling, activated stromal fibroblasts, and cysteine cathepsins B and L drive the invasive growth of human melanoma cells. Am J Pathol. 2012;181:2202–16.
74 Kasabova M, Joulin-Giet A, Lecaille F, Gilmore BF, Marchand-Adam S, Suidi A, et al. Regulation of TGF-β1-driven differentiation of human liver fibroblasts: emerging roles of cathepsin B and cystatin C. J Biol Chem. 2014;289:16239–51.
75 Kobinski JE, Ahram M, Sloane BF. Unraveling the role of proteases in cancer. Clin Chim Acta. 2000;291:113–35.
76 Gogineni VR, Gupta R, Nalla AK, Velpula KK, Rao JS. uPAR and cathepsin B shRNA impedes TGF-β1-driven proliferation and invasion of menigoma cells in a XIAP-dependent pathway. Cell Death Dis. 2012;3:e439.
77 Glogowska A, Stetefeld J, Weber E, Ghavami S, Hoang-Vu C, Klonisch T. Epidermal growth factor cytoplasmic domain affects ErbB protein degradation by the lysosomal and ubiquitin-proteasome system in human cancer cells. Neoplasia. 2012;14:396–409.
78 Authier F, Métioui M, Bell AW, Mort JS. Negative regulation of epidermal growth factor signaling by selective proteolytic mechanisms in the endosome mediated by cathepsin B. J Biol Chem. 1999;274:33723–31.
79 Gopinathan A, DeNicola GM, Frese KK, Cook N, Karreth FA, Mayerle J, et al. Cathepsin B promotes the progression of pancreatic ductal adenocarcinoma in mice. Gut. 2012;61:877–84.
80 Alapati K, KesanaKurti D, Rao JS, Dasari VR. uPAR and cathepsin B-mediated compartmentalization of JNK regulates the migration of glioma-initiating cells. Stem Cell Res. 2014;12:716–29.
81 Mallal R, Gopinath S, Alapati K, Gondi CS, Gujrati M, Dinh DH, et al. Downregulation of uPAR and cathepsin b induces apoptosis via regulation of Bcl-2 and Bax and inhibition of the PI3K/Akt pathway in gliomas. PLoS One. 2010;5:e13731.
82 Bian B, Mongrain S, Cagnol S, Langlois M-J, Boulanger J, Bernatchez G, et al. Cathepsin B promotes colorectal tumorigenesis, cell invasion, and metastasis. Mol Carcinog. 2016;55:671–87.
83 Hiwasa T, Sakiyama S, Yokoyama S, Ha J-M, Fujita J, Noguchi S, et al. Inhibition of cathepsin L-induced degradation of epidermal growth factor receptors by c-Ha-ras gene products. Biochem Biophys Res Commun. 1988;151:78–85.
84 Dennenmärker J, Lohmüller T, Mayerle J, Tacke M, Lerch MM, Coussens LM, et al. Deficiency for the cysteine protease cathepsin L promotes tumor progression in mouse epidermis. Oncogene. 2010;29:1611–21.
85 Pranmol MZI, Gutowski NJ, Hannemann M, Whatmore JL. Cathepsin L induces proangiogenic changes in human omental microvascular endothelial cells via activation of the ERK1/2 pathway. Curr Cancer Drug Targets. 2019;19:231–42.
86 Wang Z, Xiang Z, Zhu T, Chen J, Zhong M-Z, Huang J, et al. Cathepsin L interacts with CDK2-AP1 as a potential predictor of prognosis in patients with breast cancer. Oncol Lett. 2020;19:167–76.
87 Zhou W, Guan X, Song L, Liao Y, Huang J. p12 (CDK2-AP1) inhibits breast cancer cell proliferation and in vivo tumor growth. J Cancer Res Clin Oncol. 2012;138:2085–93.
88 Goulet B, Truscott M, Nepveu A. A novel proteolytically processed CDP/Cux isoform of 90 kDa is generated by cathepsin L. J Biol Chem. 2006;387:1285–93.
89 Goulet B, Sansregret L, Leduy L, Bogyo M, Weber E, Chauhan SS, et al. Increased expression and activity of nuclear cathepsin L in cancer cells suggests a novel mechanism of cell transformation. Mol Cancer Res. 2007;5:899–907.
90 Sudhan DR, Siemmn D. Cathepsin L targeting in cancer treatment. Pharmacol Ther. 2015;155:105–16.
91 Kesinger V, Sansregret L, Harada R, Vadnais C, Cadieux C, Fathers K, et al. p110 CUX1 homeodomain protein stimulates cell migration and invasion in part through a regulatory cascade culminating in the repression of E-cadherin and occludin. J Biol Chem. 2009;284:27701–11.
92 Burton LJ, Hawsawi O, Sweeney J, Bowen N, Hudson T, Odero-Marah V. CCAAT-displacement protein/cut homeobox transcription factor (CUX1) represses estrogen receptor-alpha (ER-α) in triple-negative breast cancer cells and can be antagonized by muscadine grape skin extract (MSKE). PLoS One. 2019;14:e0214844.
Peptidases in cancer and neurodegeneration

93 Grotsky DA, Gonzalez-Suarez I, Novell A, Neumann MA, Yaddanapudi SC, Croke M, et al. BRCA1 loss activates cathepsin L-mediated degradation of 53BP1 in breast cancer cells. J Cell Biol. 2013;200:187–202.

94 Al-Hashimi A, Venugopalan V, Sereesongsaeng N, Tedelind S, Pinzaru AM, Hein Z, et al. Significance of nuclear cathepsin V in normal thyroid epithelial and carcinoma cells. Biochim Biophys Acta Mol Cell Res. 2020;1867:118846.

95 Sereesongsaeng N, McDowell SH, Burrows JF, Scott CJ, Burden RE. Cathepsin V suppresses GATA3 protein expression in luminal A breast cancer. Breast Cancer Res. 2020;22:1–12.

96 Huang C-C, Chen K-L, Cheung CHA, Chang J-Y. Autophagy induced by cathepsin S inhibition induces early ROS production, oxidative DNA damage, and cell death via xanthine oxidase. Free Radiol Biol Med. 2013;65:1473–86.

97 Zhang L, Wang H, Xu J, Zhu J, Ding K. Inhibition of cathepsin S induces autophagy and apoptosis in human glioblastoma cell lines through ROS-mediated PI3K/AKT/mTOR/p70S6K and JNK signaling pathways. Toxicol Lett. 2014;228:248–59.

98 Fei M, Zhang L, Wang H, Zhu Y, Niu W, Tang T, et al. Inhibition of cathepsin S induces mitochondrial apoptosis in glioblastoma cell lines through mitochondrial stress and autophagosome accumulation. Front Oncol. 2020;10:516746.

99 Chen K-L, Chang W-SW, Cheung CHA, Lin C-C, Huang C-C, Yang Y-N, et al. Targeting cathepsin S induces tumor cell autophagy via the EGFR–ERK signaling pathway. Cancer Lett. 2012;317:89–98.

100 Yuan Y, Xue L, Fan H. Screening of differentially expressed genes related to esophageal squamous cell carcinoma and functional analysis with DNA microarrays. Int J Oncol. 2014;44:1163–70.

101 Jiang H, Wu Cheng X, Shi G-P, Hu L, Inoue A, Yamamura Y, et al. Cathepsin K-mediated notch1 activation contributes to neovascularization in response to hypoxia. Nat Commun. 2014;5:3838.

102 Verbovšek U, Motaln H, Rotter A, Atai NA, Gruden K, Van Noorden CJF, et al. Expression analysis of all protease genes reveals cathepsin K to be overexpressed in glioblastoma. PLoS One. 2014;9:e111819.

103 Hira VV, Ploegmakers KJ, Grevers F, Verbovšek U, Silvestre-Roig C, Aronica E, et al. CD133 + and nestin + glioma stem-like cells reside around CD31 + arterioles in niches that express SDF-1α, CXCR4, osteopontin and cathepsin K. J Histochem Cytochem. 2015;63:481–93.

104 Kollet O, Dar A, Shvitel S, Kalinkovich A, Lapid K, Sztainberg Y, et al. Osteoclasts degrade endosteal components and promote mobilisation of hematopoietic progenitor cells. Nat Med. 2006;12:657–64.

105 Zhang G-P, Yue X, Li S-Q. Cathepsin C interacts with TNF-α/p38 MAPK signaling pathway to promote proliferation and metastasis in hepatocellular carcinoma. Cancer Res Treat. 2020;52:10–23.

106 Kraus S, Fruth M, Bunsen T, Nägler D. IGFl receptor phosphorylation is impaired in cathepsin X-deficient prostate cancer cells. Biol Chem. 2012;393:1457–62.

107 Kos J, Vizin T, Fonović UP, Pšil A. Intraacellular signaling by cathepsin X: molecular mechanisms and diagnostic and therapeutic opportunities in cancer. Semin Cancer Biol. 2015;31:76–83.

108 Li W, Yu X, Ma X, Xie L, Xia Z, Liu L, et al. Deguelin attenuates non-small cell lung cancer cell metastasis through inhibiting the CisZ/FAK signaling pathway. Cell Signal. 2018;50:131–41.

109 Lin Y, Liao K, Miao Y, Qian Z, Fang Z, Yang X, et al. Role of asparagine endopeptidase in mediating wild-type p53 inactivation of glioblastoma. J Natl Cancer Inst. 2020;112:343–55.

110 Hallal S, Mallawaaratrathy DM, Wei H, Ebrahimkhani S, Stringer BW, Day BW, et al. Extracellular vesicles released by glioblastoma cells stimulate normal astrocytes to acquire a tumor-supportive phenotype Via p53 and MYC signaling pathways. Mol Neurobiol. 2019;56:4566–81.

111 Kang L, Shen L, Lu W, Wang D, Zhao Y, Chen C, et al. Asparaginyl endopeptidase induces endothelial permeability and tumor metastasis via downregulating zonula occludens protein ZO-1. Biochim Biophys Acta - Mol Basis Dis. 2019;1865:2267–75.

112 Liu Y, Bajuri KM, Liu C, Sinha SC. Targeting cell surface alpha(v)beta(3) integrin increases therapeutic efficacies of a legumain protease-activated auristatin prodrug. Mol Pharm. 2012;9:168–75.

113 Li X, Tang M, Zhu Q, Wang X, Lin Y, Wang X. The exosomal integrin α5β1/AEP complex derived from epithelial ovarian cancer cells promotes peritoneal metastasis through regulating mesothelial cell proliferation and migration. Cell Oncol (Dordr). 2020;43:263–77.

114 Pranjol ZI, Whatmore JL. Cathepsin D in the tumor microenvironment of breast and ovarian cancers. In: Birbrair A, editor. Tumor microenvironment: molecular players – part A. Cham: Springer International Publishing; 2020. p. 1–16.

115 Faridi JS, Mohan S, De León DD. Modulation of cathepsin D routing by IGF-II involves IGF-II binding to IGF-II/M6P receptor in MCF-7 breast cancer cells. Growth Factors. 2004;22:169–77.

116 Pranjol MZI, Gutowski NJ, Hanemann M, Whatmore JL. Cathepsin D non-proteolytically induces proliferation and migration in human omental microvascular endothelial cells via activation of the
ERK1/2 and PI3K/AKT pathways. Biochim Biophys Acta Mol Cell Res. 2018;1865:25–33.
117 Laurent-Matha V, Maruani-Herrmann S, Prébois C, Beaujouin M, Gloudu M, Noël A, et al. Catalytically inactive human cathepsin D triggers fibroblast invasive growth. J Cell Biol. 2005;168:489–99.
118 Deroç D, Prébois C, Beaujouin M, Laurent-Matha V, Pattingre S, Smith GK, et al. Cathepsin D is partly endocytosed by the LR1P receptor and inhibits LR1P-regulated intramembrane proteolysis. Oncogene. 2012;31:3202–12.
119 Wilson TJ, Namuru KC, Singh RK. Cathepsin G-mediated activation of pro-matrix metalloproteinase 9 at the tumor-bone interface promotes transforming growth factor-beta signaling and bone destruction. Mol Cancer Res. 2009;7:1224–33.
120 Morimoto-Kamata R, Yui S. Insulin-like growth factor-1 signaling is responsible for cathepsin G-induced aggregation of breast cancer MCF-7 cells. Cancer Sci. 2017;108:1574–83.
121 Morimoto-Kamata R, Tsuji D, Yui S. Cathepsin G-induced insulin-like growth factor (IGF) elevation in MCF-7 medium is caused by proteolysis of IGF binding protein (IGFBP)-2 but not of IGF-1. Biol Pharm Bull. 2020;43:1678–86.
122 Vasiljeva O, Turb B. Dual contrasting roles of cysteine cathepsins in cancer progression: apoptosis versus tumour invasion. Biochimie. 2008;90:380–6.
123 Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. Nat Rev Mol Cell Biol. 2008;9:231–41.
124 Cirman T, Oresić K, Mazovec GD, Turk V, Reed JC, Myers RM, et al. Selective disruption of lysosomes in hela cells triggers apoptosis mediated by cleavage of bid by multiple papain-like lysosomal cathepsins. J Biol Chem. 2004;279:3578–87.
125 Droga-Mazovec G, Bojić L, Petelin A, Ivanova S, Romih R, Repnik U, et al. Cysteine cathepsins trigger caspase-dependent cell death through cleavage of bid and antiapoptotic Bcl-2 homologues. J Biol Chem. 2008;283:19140–50.
126 Stoka V, Turb B, Schendel SL, Kim T-H, Cirman T, Snipas SJ, et al. Lysosomal protease pathways to apoptosis. J Biol Chem. 2001;276:3149–57.
127 Soond SM, Savvatveeva LV, Makarov VA, Gorokhvets NV, Townsend PA, Zamyatnin AA. Cathepsin S cleaves BAX as a novel and therapeutically important regulatory mechanism for apoptosis. Pharmaceutics. 2021;13:339.
128 Taha TA, Kitatani K, Bielawski J, Cho W, Hannun YA, Obeid LM. Tumor necrosis factor induces the loss of sphingosine kinase-1 by a cathepsin B-dependent mechanism. J Biol Chem. 2005;280:17196–202.
129 McComb S, Shutinoski B, Thurston S, Cessford E, Kumar K, Sad S. Cathepsins limit macrophage necroptosis through cleavage of Rip1 kinase. J Immunol. 2014;192:5671–8.
130 Nagakannan P, Islam M, Conrad M, Eftekharpour E. Cathepsin B is an executioner of ferroptosis. Biochim Biophys Acta Mol Cell Res. 2021;1868:118928.
131 Jean D, Hermann J, Rodrigues-Lima F, Barel M, Balbo M, Frade R. Identification on melanoma cells of p39, a cysteine proteinase that cleaves C3, the third component of complement: amino-acid-sequence identities with procathepsin L. Biochem J. 1995;312:961–9.
132 Seo SU, Woo SM, Min K, Kwon TK. Z-FL-COCHO, a cathepsin S inhibitor, enhances oxaliplatin-induced apoptosis through upregulation of Bim expression. Biochem Biophys Res Commun. 2018;498:849–54.
133 Khakht TP, Singh MP, Khan I, Bhardwaj M, Kang SC. Targeting of cathepsin C induces autophagic dysregulation that directs ER stress mediated cellular cytotoxicity in colorectal cancer cells. Cell Signal. 2018;46:92–102.
134 Teller A, Jechorek D, Hartig R, Adolf D, Reißig K, Roessner A, et al. Dysregulation of apoptotic signaling pathways by interaction of RPLP0 and cathepsin X/Z in gastric cancer. Pathol Res Pract. 2015;211:62–70.
135 Zhang J, Lin Y, Hu X, Wu Z, Guo W. VPS52 induces apoptosis via cathepsin D in gastric cancer. J Mol Med. 2017;95:1107–16.
136 Hah Y-S, Noh HS, Ha JH, Ahn JS, Hahn JR, Cho HY, et al. Cathepsin D inhibits oxidative stress-induced cell death via activation of autophagy in cancer cells. Cancer Lett. 2012;323:208–14.
137 Zheng W, Chen Q, Wang C, Yao D, Zhu L, Pan Y, et al. Inhibition of Cathepsin D (CTSD) enhances radiosensitivity of glioblastoma cells by attenuating autophagy. Mol Carcinog. 2020;59:651–60.
138 Woo SM, Min K-J, Seo SU, Kim S, Park J-W, Song DK, et al. Up-regulation of 5-lipoxygenase by inhibition of cathepsin G enhances TRAIL-induced apoptosis through down-regulation of survivin. Oncotarget. 2017;8:106672–84.
139 Kramer L, Turk D, Turk B. The future of cysteine cathepsins in disease management. Trends Pharmacol Sci. 2017;38:873–98.
140 Aggarwal N, Sloane BF. Cathepsin B: multiple roles in cancer. Proteomics Clin Appl. 2014;8:427–37.
141 Garcia-Cattaneo A, Gobert F-X, Muller M, Toscano F, Flores M, Lescure A, et al. Cleavage of Toll-like receptor 3 by cathepsins B and H is essential for transition of tumor cells. Eur J Cell Biol. 2017;96:622–31.
142 Mitrović A, Pećar Fonović U, Kos J. Cysteine cathepsins B and X promote epithelial-mesenchymal transition of tumor cells. Expert Opin Ther Targets. 2013;17:281–91.
144 Son H, Moon A. Epithelial-mesenchymal transition and cell invasion. Toxicol Res. 2010;26:245–52.
145 Goecheva V, Zeng W, Ke D, Klimstra D, Reinheckel T, Peters C, et al. Distinct roles for cysteine cathepsin genes in multistage tumorigenesis. Genes Dev. 2006;20:543–56.
146 Fonović M, Turk B. Cysteine cathepsins and extracellular matrix degradation. Biochim Biophys Acta - Gen Subj. 2014;1840:2560–70.
147 Zhang Q, Han M, Wang W, Song Y, Chen G, Wang Z, et al. Downregulation of cathepsin L suppresses cancer invasion and migration by inhibiting transforming growth factor-β-mediated epithelial-mesenchymal transition. Oncol Rep. 2015;33:1851–9.
148 Wang L, Zhao Y, Xiong Y, Wang W, Fei Y, Tan C, et al. K-ras mutation promotes ionizing radiation-induced invasion and migration of lung cancer in part via the Cathepsin L/CUX1 pathway. Exp Cell Res. 2018;362:424–35.
149 Fei Y, Xiong Y, Shen X, Zhao Y, Zhu Y, Wang L, et al. Cathepsin L promotes ionizing radiation-induced U251 glioma cell migration and invasion through regulating the GSK-3β/CUX1 pathway. Cell Signal. 2018;44:62–71.
150 Yu S, Yu Y, Zhang W, Yuan W, Zhao N, Li Q, et al. FOXO3a promotes gastric cancer cell migration and invasion through the induction of cathepsin L. Oncotarget. 2016;7:34773–84.
151 Xiong Y, Ji W, Fei Y, Zhao Y, Wang L, Wang W, et al. Cathepsin L is involved in X-ray-induced invasion and migration of human glioma U251 cells. Cell Signal. 2017;29:181–91.
152 Wang C-H, Wang L-K, Wu C-C, Chen M-L, Kuo C-Y, Shyu R-Y, et al. Cathepsin V mediates the tazarotene-induced gene 1-induced reduction in invasion in colorectal cancer cells. Cell Biochem Biophys. 2020;78:483–94.
153 Yasuda Y, Li Z, Greenbaum D, Bogoy M, Weber E, Brömme D. Cathepsin V, a novel and potent elastolytic activity expressed in activated macrophages. J Biol Chem. 2004;279:36761–70.
154 Zhang L, Wang H, Xu J Cathepsin S as a cancer target. Neoplasma. 2015;62:16–26.
155 Sevenich L, Bowman RL, Mason SD, Quail DF, Rapaport F, Elie BT, et al. Analysis of tumour- and stroma-supplied proteolytic networks reveals a brain-metastasis-promoting role for cathepsin S. Nat Cell Biol. 2014;16:867–88.
156 Wei L, Shao N, Peng Y, Zhou P. Inhibition of cathepsin S restores TGF-β-induced epithelial-to-mesenchymal transition and tight junction turnover in glioblastoma cells. J Cancer. 2021;12:1592–603.
157 Jevnikar Z, Rojnik M, Jamnik P, Doljak B, Fonovic UP, Kos J. Cathepsin H mediates the processing of talin and regulates migration of prostate cancer cells. J Biol Chem. 2013;288:2201–9.
158 Verbovšek U, Van Noorden CJF, Lah TT. Complexity of cancer protease biology: cathepsin K expression and function in cancer progression. Semin Cancer Biol. 2015;35:71–84.
159 Podgorski I, Linebaugh BE, Slaone BF. Cathepsin K in the bone microenvironment: link between obesity and prostate cancer? Biochem Soc Trans. 2007;35:701–3.
160 Lechner AM, Assfalq-Muchleitl I, Zahler S, Stoeckelhuber M, Machleitd W, Jochum M, et al. RGD-dependent binding of procathepsin X to integrin α vβ3 mediates cell-adhesive properties. J Biol Chem. 2006;281:39588–97.
161 Lines KE, Chelala C, Dmitrovic B, Wijesuriya N, Kocher HM, Marshall JF, et al. S100P-binding protein, S100PBP, mediates adhesion through regulation of cathepsin Z in pancreatic cancer cells. Am J Pathol. 2012;180:1485–94.
162 Pečar Fonović U, Jevnikar Z, Rojnik M, Doljak B, Fonović M, Jamnik P, et al. Profilin 1 as a target for cathepsin X activity in tumor cells. PLoS One. 2013;8:e53918.
163 Vasiljeva O, Papazoglou A, Kräger A, Brodoeffel H, Korovin M, Deussing J, et al. Tumor cell-derived and macrophage-derived cathepsin B promotes progression and lung metastasis of mammary cancer. Cancer Res. 2006;66:5242–50.
164 Sevenich L, Schurigt U, Sachse K, Gajda M, Werner F, Müller S, et al. Synergistic antitumor effects of combined cathepsin B and cathepsin Z deficiencies on breast cancer progression and metastasis in mice. Proc Natl Acad Sci USA. 2010;107:2497–502.
165 Kraus S, Bunsen T, Schuster S, CichóI MA, Tacke M, Reinheckel T, et al. Cellular senescence induced by cathepsin X downregulation. Eur J Cell Biol. 2011;90:678–86.
166 Wang J, Chen L, Li Y, Guan X-Y. Overexpression of cathepsin Z contributes to tumor metastasis by inducing epithelial-mesenchymal transition in hepatocellular carcinoma. PLoS One. 2011;6:e24967.
167 Lunde NN, Bosnjak T, Solberg R, Johansen HT. Mammalian legumain – a lysosomal cysteine protease with extracellular functions? Biochimie. 2019;166:77–83.
168 Chen JM, Fortunato M, Stevens RA, Barrett AJ. Activation of progelatinase A by mammalian legumain, a recently discovered cysteine proteinase. Biol Chem. 2001;382:777–83.
169 Shirahama-Noda K, Yamamoto A, Sugihara K, Hashimoto N, Asano M, Nishimura M, et al. Biosynthetic Processing of cathepsins and lysosomal degradation are abolished in asparaginyl endopeptidase-deficient mice. J Biol Chem. 2003;278:33194–9.
170 Wang Y, Zhang S, Wang H, Cui Y, Wang Z, Cheng X, et al. High level of legumain was correlated with worse prognosis and peritoneal metastasis in gastric cancer patients. Front Oncol. 2020;10:966.

171 Maynadier M, Farnoud R, Lamy P-J, Laurent-Matha V, Garcia M, Rochefort H. Cathepsin D stimulates the activities of secreted plasminogen activators in the breast cancer acidic environment. Int J Oncol. 2013;43:1683–90.

172 Zeleznik TZ, Kadin A, Turk V, Dolenc I. Aspartic cathepsin D degrades the cytosolic cysteine cathepsin inhibitor stefin B in the cells. Biochem Biophys Res Commun. 2015;465:213–7.

173 Laurent-Matha V, Huesgen PF, Masson O, Deroçq D, Prébois C, Gary-Bobo M, et al. Proteolysis of cystatin C by cathepsin D in the breast cancer microenvironment. FASEB J. 2012;26:5172–81.

174 Vangala G, Imhoff FM, Squires CML, Criddle AG, Baird SK. Mesenchymal stem cell homing towards cancer cells is increased by enzyme activity of cathepsin D. Exp Cell Res. 2019;383:111494.

175 Kawakubo T, Yasukochi A, Toyama T, Takahashi S, Okamoto K, Tsukuba T, et al. Repression of cathepsin E expression increases the risk of mammary carcinogenesis and links to poor prognosis in breast cancer. Carcinogenesis. 2014;35:714–26.

176 Kawakubo T, Okamoto K, Iwata J, Shin M, Okamoto Y, Yasukochi A, et al. Cathepsin E prevents tumor growth and metastasis by catalyzing the proteolytic release of soluble TRAIL from tumor cell surface. Cancer Res. 2007;67:10869–78.

177 Yasukochi A, Kawakubo T, Nakamura S, Yamamoto K. Cathepsin E enhances anticancer activity of doxorubicin on human prostate cancer cells showing resistance to TRAIL-mediated apoptosis. Biol Chem. 2010;391:947–58.

178 Yui S, Tomita K, Kudo T, Ando S, Yamazaki M. Induction of multicellular 3-D spheroids of MCF-7 breast carcinoma cells by neutrophil-derived cathepsin G and elastase. Cancer Sci. 2005;96:560–70.

179 Kudo T, Kigoshi H, Hagiwara T, Takino T, Yamazaki M, Yui S, Cathepsin G, a neutrophil protease, induces compact cell-cell adhesion in MCF-7 human breast cancer cells. Mediators Inflamm. 2009;2009:850940.

180 Vanderboor CMG, Thibeault PE, Nixon KCJ, Gros R, Kramer J, Ramachandran R. Protease-activated receptor 4 activation triggers cell membrane blebbing through RhoA and β-arrestin. Mol Pharmacol. 2020;97:365–76.

181 De Palma M, Biziato D, Petrova TV. Microenvironmental regulation of tumour angiogenesis. Nat Rev Cancer. 2017;17:457–74.

182 Kostoulas G, Lang A, Nagase H, Baici A. Stimulation of angiogenesis through cathepsin B inactivation of the tissue inhibitors of matrix metalloproteinases. FEBS Lett. 1999;455:286–90.

183 Urbich C, Heeschen C, Aicher A, Sasaki K, Bruhl T, Farhadi MR, et al. Cathepsin L is required for endothelial progenitor cell–induced neovascularization. Nat Med. 2005;11:206–13.

184 Wang B, Sun J, Kitamoto S, Yang M, Grubb A, Chapman HA, et al. Cathepsin S controls angiogenesis and tumor growth via matrix-derived angiogenic factors. J Biol Chem. 2006;281:6020–9.

185 Gocheva V, Chen X, Peters C, Reinheckel T, Joyce JA. Deletion of cathepsin H perturbs angiogenic switching, vascularization and growth of tumors in a mouse model of pancreatic islet cell cancer. Biol Chem. 2010;391:937–45.

186 Ohri SS, Vashishta A, Proctor M, Fusek M, Vetvicka V. The propeptide of cathepsin D increases proliferation, invasion and metastasis of breast cancer cells. Int J Oncol. 2008;32:491–8.

187 Briozzo P, Badet J, Caponi F, Pieri I, Montcourrier P, Barritault D, et al. MCF7 mammary cancer cells respond to bFGF and internalize it following its release from extracellular matrix: a permissive role of cathepsin D. Exp Cell Res. 1991;194:252–9.

188 Jha SK, Rauniar HR, Chronowska E, Mattonet K, Maina EW, Koistinen H, et al. KLK3/PSA and cathepsin D activate VEGF-C and VEGF-D. Elife. 2019;8:e44478.

189 Shin M, Kadowaki T, Iwata J, Kawakubo T, Yamaguchi N, Takii R, et al. Association of cathepsin E with tumor growth arrest through angiogenesis inhibition and enhanced immune responses. Biol Chem. 2007;388:1173–81.

190 Wilson TJ, Nannuru KC, Futakuchi M, Singh RK. Cathepsin G-mediated enhanced TGF-beta signaling promotes angiogenesis via upregulation of VEGF and MCP-1. Cancer Lett. 2010;288:162–9.

191 Uzhchenko RV, Shanker A. CD8+ T lymphocyte and NK cell network: circuits in the cytotoxic domain of immunity. Front Immunol. 2019;10:1906.

192 Jewett A, Kos J, Fong Y, Ko M-W, Safaei T, Peri Uzhachenko RV, Shanker A. CD8 T lymphocyte and NK cell network: circuitry in the cytotoxic domain of immunity. Front Immunol. 2019;10:1906.

193 Kaur K, Cook J, Park S-H, Topchyan P, Kozlowska A, Ohanian N, et al. Novel strategy to expand supercharged NK cells with significant potential to lyse and differentiate cancer stem cells: differences in NK expansion and function between healthy and cancer patients. Front Immunol. 2017;8:297.

194 Voskoboinik I, Whistock JC, Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol. 2015;15:388–400.
195 Martínez-Lostao L, Anel A, Pardo J. How do cytotoxic lymphocytes kill cancer cells? *Clin Cancer Res.* 2015;21:5047–56.

196 Nanek O, Avcín T, Bedina Zavec A. Perforin and human diseases. *Subcell Biochem.* 2014;80:221–39.

197 Konjar S, Sutton VR, Hoves S, Repnik U, Yagita H, Reinheckel T, et al. Human and mouse perforin are processed in part through cleavage by the lysosomal cysteine proteinase cathepsin L. *Immunology.* 2010;131:257–67.

198 Masson D, Tschopp J. A family of serine esterases in lytic granules of cytolytic T lymphocytes. *Cell.* 1987;49:679–85.

199 Sutton VR, Waterhouse NJ, Browne KA, Sedelies K, Ciccone A, Anthony D, et al. Residual active granzyme B in cathepsin C–null lymphocytes is sufficient for perforin-dependent target cell apoptosis. *J Cell Biol.* 2007;176:425–33.

200 D’Angelo ME, Bird PI, Peters C, Reinheckel T, Trapani JA, Sutton VR. Cathepsin H is an additional convertase of pro-granzyme B. *J Biol Chem.* 2010;285:20514–9.

201 Alvarez-Fernandez M, Barrett AJ, Gerhartz B, Dando PM, Ni J, Abrahamson M. Inhibition of mammalian legumain by some cystatins is due to a novel second reactive site. *J Biol Chem.* 1999;274:19195–203.

202 Colbert JD, Plechanovová A, Watts C. Glycosylation directs targeting and activation of cystatin f from intracellular and extracellular sources. *Traffic.* 2009;10:425–37.

203 Hamilton G, Colbert JD, Schuettelkopf AW, Watts C. Cystatin F is a cathepsin C-directed protease inhibitor regulated by proteolysis. *EMBO J.* 2008;27:499–508.

204 Magister S, Obermajer N, Mirković B, Švaiger U, Renko M, Softić A, et al. Regulation of cathepsins S and L by cystatin F during maturation of dendritic cells. *Eur J Cell Biol.* 2012;91:391–401.

205 Colbert JD, Matthews SP, Kos J, Watts C. Internalization of exogenous cystatin F suppresses cysteine proteinase activates and reduces the accumulation of single-chain cathepsin L by multiple mechanisms. *J Biol Chem.* 2011;286:42082–90.

206 Magister S, Tseng H-C, Bui VT, Kos J, Jewett A. Regulation of split anergy in natural killer cells by inhibition of cathepsins C and H and cystatin F. *Oncotarget.* 2015;6:22310–27.

207 Prunk M, Perišić Nanut M, Sabotić J, Švaiger U, Kos J. Increased cystatin F levels correlate with decreased cytotoxicity of cytotoxic T cells. *Radiol Oncol.* 2019;53:57–68.

208 Perišić Nanut M, Pauwelec G, Kos J. Human CD4+ T-cell clone expansion leads to the expression of the cysteine peptidase inhibitor cystatin F. *Int J Mol Sci.* 2021;22:8408.

209 Puxbaum V. Proteinases and their inhibitors in liver cancer. *World J Hepatol.* 2009;1:28.

210 Utsunomiya T, Hara Y, Kataoka A, Morita M, Arakawa H, Mori M, et al. Cystatin-like metastasis-associated protein mRNA expression in human colorectal cancer is associated with both liver metastasis and patient survival. *Clin Cancer Res.* 2002;8:2591–4.

211 Senjur E, Perišić Nanut M, Breznik B, Mitrović A, Mlakar J, Rotter A, et al. Cystatin F acts as a mediator of immune suppression in glioblastoma. *Cell Oncol.* 2021;44:1051–63.

212 Bowman RL, Wang Q, Carro A, Verhaak RGW, Squatrito M. GlioVis data portal for visualization and analysis of brain tumor expression datasets. *Neuro Oncol.* 2017;19:139–41.

213 Prunk M, Nanut MP, Jakos T, Sabotić J, Švaiger U, Kos J. Extracellular cystatin F is internalised by cytotoxic T lymphocytes and decreases their cytotoxicity. *Cancers (Basel).* 2020;12:1–14.

214 Schittenhelm L, Hilkens CM, Morrison VL. β2 Integrins as regulators of dendritic cell, monocyte, and macrophage function. *Front Immunol.* 2017;8:1866.

215 Arbore G, West EE, Rahman J, Le Fricc G, Niyonzima N, Pirooznia M, et al. Complement receptor CD46 co-stimulates optimal human CD8+ T cell effector function via fatty acid metabolism. *Nat Commun.* 2018;9:4186.

216 Liszewski MK, Kolev M, Le Fricc G, Leung M, Bertram PG, Fara AF, et al. Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. *Immunity.* 2013;39:1143–57.

217 Freeley S, Cardone J, Günther SC, West EE, Reinheckel T, Watts C, et al. Asparaginyl endopeptidase (Legumain) supports human Th1 induction via cathepsin L-mediated intracellular C3 activation. *Front Immunol.* 2018;9:2449.

218 Bailey SR, Nelson MH, Himes RA, Li Z, Mehrotra S, Paulos CM. Th17 cells in cancer. The ultimate identity crisis. *Front Immunol.* 2014;5:276.

219 Salkowska A, Karam K, Karwaciak I, Walczak-Drzewiecka A, Krawczyk M, Sobalska-Kwapis M, et al. Identification of novel molecular markers of human Th17 cells. *Cells.* 2020;9:1611.

220 Hou L, Cooley J, Swanson R, Ong PC, Pike RN, Bogyo M, et al. The protease cathepsin L regulates Th17 cell differentiation. *J Autoimmun.* 2015;65:56–63.

221 Dekita M, Wu Z, Ni J, Zhang X, Liu Y, Yan X, et al. Cathepsin S Is Involved in Th17 differentiation through the upregulation of IL-6 by activating PAR-2 after systemic exposure to lipopolysaccharide from porphyromonas gingivalis. *Front Pharmacol.* 2017;8:470.

222 Hatziouannou A, Boumpas A, Papadopoulou M, Papafragkos I, Varveri A, Alissafi T, et al. Regulatory
T cells in autoimmunity and cancer: a duplicitous lifestyle. *Front Immunol*. 2021;12:731947.

223 Yan X, Wu C, Chen T, Santos MM, Liu C-L, Yang C, et al. Cathepsin S inhibition changes regulatory T-cell activity in regulating bladder cancer and immune cell proliferation and apoptosis. *Mol Immunol*. 2017;82:66–74.

224 Zhou Y, Chen H, Liu L, Yu X, Sukhova GK, Yang M, et al. Cathepsin K deficiency ameliorates systemic lupus erythematosus-like manifestations in fas lpr mice. *J Immunol*. 2017;198:1846–54.

225 Zhang Q, Liu L, Gong C, Shi H, Zeng Y, Wang X, et al. Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PLoS One*. 2012;7:e50946.

226 Pan Y, Yu Y, Wang X, Zhang T. Tumor-associated macrophages in tumor immunity. *Front Immunol*. 2020;11:583084.

227 Millrud CR, Bergenfelz C, Leandersson K. On the origin of myeloid-derived suppressor cells. *Oncotarget*. 2017;8:3649–65.

228 Cui TX, Kryczek I, Zhao L, Zhao E, Kuick R, Roh MH, et al. Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing MicroRNA101 and suppressing the corepressor CtBP2. *Immunity*. 2013;39:611–21.

229 Wan S, Zhao E, Kryczek I, Vatan L, Sadovskaya A, Ludema G, et al. Tumor-associated macrophages produce interleukin 6 and signal via STAT3 to promote expansion of human hepatocellular carcinoma stem cells. *Gastroenterology*. 2014;147:1393–404.

230 Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer Immunol Res*. 2017;5:3–8.

231 Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med*. 2013;19:1423–37.

232 Krueger S, Kalinski T, Wolf H, Kellner U, Roessner A. Interactions between human colon carcinoma cells, fibroblasts and monocytic cells in coculture—regulation of cathepsin B expression and invasiveness. *Cancer Lett*. 2005;223:313–22.

233 Jakos T, Pilhar A, Pečar Fonović U, Kos J. Lysosomal peptidases in innate immune cells: implications for cancer immunity. *Cancer Immunol Immunother*. 2020;69:275–83.

234 Bourré AM, Friedman DB, Bogyo M, Min Y, Yang L, Lin PC. Identification of a myeloid-derived suppressor cell cystatin-like protein that inhibits metastasis. *FASEB J*. 2011;25:2626–37.

235 Akkari L, Gocheva V, Kester JC, Hunter KE, Quick ML, Sevenich L, et al. Distinct functions of macrophage-derived and cancer cell-derived cathepsin Z combine to promote tumor malignancy via interactions with the extracellular matrix. *Genes Dev*. 2014;28:2134–50.

236 Gounaris E, Tung CH, Restaino C, Maehr R, Kohler R, Joyce JA, et al. Live imaging of cysteine-cathepsin activity reveals dynamics of focal inflammation, angiogenesis, and polyp growth. *PLoS One*. 2008;3:e2916.

237 Ha S-D, Martins A, Khazaie K, Han J, Chan BMC, Kim SO. Cathepsin B is involved in the trafficking of TNF-α-containing vesicles to the plasma membrane in macrophages. *J Immunol*. 2008;181:690–7.

238 Podgorski I, Linebaugh BE, Kobinski JE, Rudy DL, Herroon MK, Olive MB, et al. Bone marrow-derived cathepsin K cleaves SPARC in bone metastasis. *Am J Pathol*. 2009;175:1255–69.

239 Herroon MK, Rajagurubandara E, Rudy DL, Chalasani A, Hardaway AL, Podgorski I. Macrophage cathepsin K promotes prostate tumor progression in bone. *Oncogene*. 2013;32:1580–93.

240 Obermajer N, Kalinski P. Generation of myeloid-derived suppressor cells using prostaglandin E2. *Transplant Res*. 2012;1:15.

241 Prima V, Kaliberova LN, Kaliberov S, Curiel DT, Kusmartsev S. COX2/mPGES1/PGE2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells. *Proc Natl Acad Sci USA*. 2017;114:1117–22.

242 Mancini A, Jovanovic DV, He QW, Di Battista JA. Site-specific proteolysis of cyclooxygenase-2: a putative step in inflammatory prostaglandin E2 biosynthesis. *J Cell Biochem*. 2007;101:425–41.

243 Wilkinson RDA, Magorrian SM, Williams R, Young A, Small DM, Scott CJ, et al. CCL2 is transcriptionally controlled by the lysosomal protease cathepsin S in a CD74-dependent manner. *Oncotarget*. 2015;6:29725–39.

244 Bruchard M, Mignot G, Derangère V, Chalmin F, Chevriaux A, Végran F, et al. Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the Nramp3 inflammasome and promotes tumor growth. *Nat Med*. 2013;19:57–64.

245 Shree T, Olson OC, Elie BT, Kester JC, Garfall AL, Simpson K, et al. Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. *Genes Dev*. 2011;25:2465–79.

246 Lie PPY, Nixon RA. Lysosome trafficking and signaling in health and neurodegenerative diseases. *Neurobiol Dis*. 2019;122:94–105.

247 Ciechanover A, Kwon YT. Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. *Exp Mol Med*. 2015;47:e147.

248 Davis AA, Leyns CEG, Holtzman DM. Intercellular spread of protein aggregates in neurodegenerative disease. *Annu Rev Cell Dev Biol*. 2018;34:545–68.

249 Glenner GG, Wong CW. Alzheimer’s disease: initial report of the purification and characterization of a
novel cerebrovascular amyloid protein. Biochem Biophys Res Commun. 1984;120:885–90.

250 Kosik KS, Joachim CL, Selkoe DJ. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci USA. 1986;83:4044–8.

251 Nixon RA, Cataldo AM, Mathews PM. The endosomal-lysosomal system of neurons in Alzheimer’s disease pathogenesis: a review. Neurochem Res. 2000;25:1161–72.

252 Nixon RA, Cataldo AM. Lysosomal system pathways: genes to neurodegeneration in Alzheimer’s disease. J Alzheimers Dis. 2006;9:277–89.

253 McDermott JR, Gibson AM. Degradation of Alzheimer’s beta-amyloid protein by human cathepsin D. NeuroReport. 1996;7:2163–6.

254 Hamazaki H. Cathepsin D is involved in the clearance of Alzheimer's beta-amyloid protein. FEBS Lett. 1996;396:139–42.

255 Ladror US, Snyder SW, Wang GT, Holzman TF, Krafft GA. Cleavage at the amino and carboxyl termini of Alzheimer’s amyloid-beta by cathepsin D. J Biol Chem. 1994;269:18422–8.

256 Kenessey A, Nacharaju P, Ko LW, Yen SH. Degradation of tau by lysosomal enzyme cathepsin D: implication for Alzheimer neurofibrillary degeneration. J Neurochem. 1997;69:2026–38.

257 Khurana V, Elson-Schwab I, Fulga TA, Sharp KA, Loewen CA, Mulkearns E, et al. Lysosomal dysfunction promotes cleavage and neurotoxicity of tau in vivo. PLoS Genet. 2010;6:e1001026.

258 Vidoni C, Follo C, Savino M, Melone MAB, Isidoro C. The role of cathepsin D in the pathogenesis of human neurodegenerative disorders. Med Res Rev. 2016;36:845–70.

259 Cataldo AM, Barnett JL, Berman SA, Li J, Quarless S, Bursztajn S, et al. Gene expression and cellular content of cathepsin D in Alzheimer’s disease brain: evidence for early up-regulation of the endosomal-lysosomal system. Neuron. 1995;14:671–80.

260 Schwagerl AL, Mohan PS, Cataldo AM, Vonsattel JP, Kowall NW, Nixon RA. Elevated levels of the endosomal-lysosomal proteinase cathepsin D in cerebrospinal fluid in Alzheimer disease. J Neurochem. 1995;64:443–6.

261 Chai YL, Chong JR, Weng J, Howlett D, Halsey A, Lee JH, et al. Lysosomal cathepsin D is upregulated in Alzheimer's disease neocortex and may be a marker for neurofibrillary degeneration. Brain Pathol. 2019;29:63–74.

262 Kim J-W, Jung S-Y, Kim Y, Heo H, Hong C-H, Seo S-W, et al. Identification of cathepsin D as a plasma biomarker for Alzheimer’s disease. Cells. 2021;10:138.

263 Zhou W, Scott SA, Shelton SB, Crutcher KA. Cathepsin D-mediated proteolysis of apolipoprotein E: possible role in Alzheimer’s disease. Neuroscience. 2006;143:689–701.

264 Nakanishi H, Amano T, Sastradipura DF, Yoshimine Y, Tsukuba T, Tanabe K, et al. Increased expression of cathepsins E and D in neurons of the aged rat brain and their colocalization with lipofuscin and carboxy-terminal fragments of Alzheimer amyloid precursor protein. J Neurochem. 1997;68:739–49.

265 Nakanishi H, Tominaga K, Amano T, Hirotsu I, Inoue T, Yamamoto K. Age-related changes in activities and localizations of cathepsins D, E, B, and L in the rat brain tissues. Exp Neurol. 1994;126:119–28.

266 Bernstein HG, Wiederanders B. An immunohistochemical study of cathepsin E in Alzheimer-type dementia brains. Brain Res. 1994;667:287–90.

267 Yamashima T. Implication of cysteine proteases calpain, cathepsin and caspase in ischemic neuronal death of primates. Prog Neurobiol. 2000;62:273–95.

268 Cataldo AM, Paskevich PA, Kominami E, Nixon RA. Lysosomal hydrolases of different classes are abnormally distributed in brains of patients with Alzheimer disease. Proc Natl Acad Sci USA. 1991;88:10998–1002.

269 Felbor U, Kessler B, Mothes W, Goebel HH, Ploegh HL, Bronson RT, et al. Neuronal loss and brain atrophy in mice lacking cathepsins B and L. Proc Natl Acad Sci USA. 2002;99:7883–8.

270 BednarSKI E, Ribak CE, Lynch G. Suppression of cathepsins B and L causes a proliferation of lysosomes and the formation of meganeurites in hippocampus. J Neurosci. 1997;17:4006–21.

271 Schechter I, Ziv E. Cathepsins S, B and L with aminopeptidases display β-secretase activity associated with the pathogenesis of Alzheimer’s disease. Biol Chem. 2011;392:555–69.

272 Hook VYH, Toneff T, Aaron W, Yasothornsrikul S, Bundey R, Reisine T. Bata-amyloid peptide in regulated secretory vesicles of chromaffin cells: evidence for multiple cysteine proteolytic activities in distinct pathways for beta-secretase activity in chromaffin vesicles. J Neurochem. 2002;81:237–56.

273 Hook G, Hook VYH, Kindy M. Cysteine protease inhibitors reduce brain beta-amyloid and beta-secretase activity in vivo and are potential Alzheimer’s disease therapeutics. Biol Chem. 2007;388:979–83.

274 Hook VYH, Kindy M, Hook G. Inhibitors of cathepsin B improve memory and reduce beta-amyloid in transgenic Alzheimer disease mice expressing the wild-type, but not the Swedish mutant, beta-secretase site of the amyloid precursor protein. J Biol Chem. 2008;283:7745–53.

275 Hook G, Hook V, Kindy M. The cysteine protease inhibitor, E64d, reduces brain amyloid-β and improves memory deficits in Alzheimer’s disease animal models.
by inhibiting cathepsin B, but not BACE1, β-secretase activity. *J Alzheimers Dis.* 2011;26:387–408.

276 Cho K, Yoon SY, Choi JE, Kang HJ, Jang HY, Kim D-H. CA-074Me, a cathepsin B inhibitor, decreases APP accumulation and protects primary rat cortical neurons treated with okadaic acid. *Neurosci Lett.* 2013;548:222–7.

277 Hook V, Funkelstein L, Wegrzyn J, Bark S, Kindy M, Hook GR. Deletion of the cathepsin B gene improves memory deficits in a transgenic ALZheimer’s disease mouse model expressing AβPP containing the wild-type β-secretase site sequence. *J Alzheimers Dis.* 2012;29:827–40.

278 Mackay EA, Ehrhard A, Moniatte M, Guenet C, Tardif C, Tarnus C, et al. A possible role for cathepsins D, E, and B in the processing of beta-amyloid precursor protein in Alzheimer’s disease. *Eur J Biochem.* 2012;279:1824:89–104.

279 Kindy MS, Yu J, Zhu H, El-Amouri SS, Hook V, Hook GR. Deletion of the cathepsin B gene improves memory deficits in a transgenic ALZheimer’s disease mouse model expressing AβPP containing the wild-type β-secretase site sequence. *J Alzheimers Dis.* 2012;29:827–40.

280 Mueller-Steiner S, Zhou Y, Arai H, Roberson ED, Sun B, Chen J, et al. Antiamyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer’s disease. *Neuron.* 2006;51:703–14.

281 Oberstein TJ, Utz J, Spitzer P, Klafki HW, Wiltfang J, Lewczuk P, et al. The role of cathepsin B in the degradation of Aβ and in the production of Aβ peptides starting with Ala2 in cultured astrocytes. *Front Mol Neurosci.* 2020;13:615740.

282 Cataldo AM, Nixon RA. Enzymatically active lysosomal proteases are associated with amyloid deposits in Alzheimer brain. *Proc Natl Acad Sci USA.* 1990;87:3861–5.

283 Nakamura Y, Takeda M, Suzuki H, Hattori H, Tada K, Hariguchi S, et al. Abnormal distribution of cathepsins in the brain of patients with Alzheimer’s disease’s neurofibrillary pathology.* Proc Natl Acad Sci USA.* 1991;98:195–8.

284 Sundelof J, Sundstrom J, Hansson O, Erkssdotter-Jonhagen M, Giedraitis V, Larsson A, et al. Higher cathepsin B levels in plasma in Alzheimer’s disease compared to healthy controls. *J Alzheimers Dis.* 2010;22:1223–30.

285 Morena F, Argentati C, Trotta R, Crispolti L, Stabile A, Pistilli A, et al. A Comparison of lysosomal enzymes expression levels in peripheral blood of mild- and severe-Alzheimer’s disease and MCI patients: implications for regenerative medicine approaches. *Int J Mol Sci.* 2017;18:1806.

286 Hook G, Yu J, Toneff T, Kindy M, Hook V. Brain pyroglutamate amyloid-β is produced by cathepsin B and is reduced by the cysteine protease inhibitor E64d, representing a potential Alzheimer’s disease therapeutic. *J Alzheimers Dis.* 2014;41:129–49.

287 Munger JS, Haass C, Lemere CA, Shi G, Wong WS, Teplow DB, et al. Lysosomal processing of amyloid precursor protein to A beta peptides: a distinct role for cathepsin S. *Biochem J.* 1995;311(PT 1):299–305.

288 Lemere CA, Munger JS, Shi GP, Nattkin L, Haass C, Chapman HA, et al. The lysosomal cysteine protease, cathepsin S, is increased in Alzheimer’s disease and Down syndrome brain. An immunocytochemical study, *Am J Pathol.* 1995;146:848–60.

289 Liuzzo JP, Petanceska SS, Devi LA. Neurotrophic factors regulate cathepsin S in macrophages and microglia: a role in the degradation of myelin basic protein and amyloid beta peptide. *Mol Med.* 1999;5:334–43.

290 Nakashishi H. Microglial functions and proteases. *Mol Neurobiol.* 2003;27:163–76.

291 Pike CJ, Burdick D, Walenczewicz AJ, Glabe CG, Cotman CW. Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J Neurosci.* 1993;13:1676–87.

292 Ii K, Ito H, Kominami E, Hirano A. Abnormal distribution of cathepsin proteinases and endogenous inhibitors (cystatins) in the hippocampus of patients with Alzheimer’s disease, parkinsonism-dementia complex on Guam, and senile dementia and in the aged. *Virchows Arch A Pathol Anat Histopathol.* 1993;423:185–94.

293 Berdowska I. Cysteine proteases as disease markers. *Clin Chim Acta.* 2004;342:41–69.

294 Wendt W, Zhu X-R, Lübbert H, Stichel CC. Differential expression of cathepsin X in aging and pathological central nervous system of mice. *Exp Neurol.* 2007;204:525–40.

295 Hafner A, Glavan G, Obermajer N, Živin M, Schliebs R, Kos J. Neuroprotective role of γ-enolase in microglia in a mouse model of Alzheimer’s disease is regulated by cathepsin X. *Aging Cell.* 2013;12:604–14.

296 Pišlar AH, Kos J. C-terminal peptide of γ-enolase impairs amyloid-β-induced apoptosis through p75 (NTR) signaling. *Neuromolecular Med.* 2013;15:623–35.

297 Tsevelevi V, Rubio R, Vamvakas S-S, White J, Taoufik E, Petit E, et al. Comparative gene expression analysis in mouse models for multiple sclerosis, Alzheimer’s disease and stroke for identifying commonly regulated and disease-specific gene changes. *Genomics.* 2010;96:82–91.

298 Zhang Z, Song M, Liu X, Kang SS, Kwon I-S, Duong DM, et al. Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer’s disease. *Nat Med.* 2014;20:1254–62.
Peptidases in cancer and neurodegeneration

Iqbal K, Liu F, Gong C-X. Tau and neurodegenerative disease: the story so far. Nat Rev Neurol. 2016;12:15–27.

FEDS Open Bio 12 (2022) 708–738 © 2022 The Authors. FEDS Open Bio published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies
activation in PC12 cells. *Biochem Pharmacol*. 2001; 62:473–81.

327 Niranjan R. The role of inflammatory and oxidative stress mechanisms in the pathogenesis of Parkinson’s disease: focus on astrocytes. *Mol Neurobiol*. 2014;49:28–38.

328 More SV, Kumar H, Kim IS, Song S-Y, Choi D-K. Cellular and molecular mediators of neuroinflammation in the pathogenesis of Parkinson’s disease. *Mediators Inflamm*. 2013;2013:952375.

329 Menza M, Dobkin RD, Marin H, Mark MH, Gara M, Bienfait K, et al. The role of inflammatory cytokines in cognition and other non-motor symptoms of Parkinson’s disease. *Psychosomatics*. 2010;51:474–9.

330 Choi D-Y, Liu M, Hunter RL, Cass WA, Pandya JD, Sullivan PG, et al. Striatal neuroinflammation promotes Parkinsonism in rats. *PLoS One*. 2009;4: e5482.

331 Nakanishi H. Microglial cathepsin B as a key driver of inflammatory brain diseases and brain aging. *Neural Regen Res*. 2020;15:25.

332 Lowry JR, Klegers A. Emerging roles of microglial cathepsins in neurodegenerative disease. *Brain Res Bull*. 2018;139:144–56.

333 Ryan RE, Sloane BF, Sameni M, Wood PL. Microglial cathepsin B: an immunological examination of cellular and secreted species. *J Neurochem*. 2002;65:1035–45.

334 Petanceska S, Canoll P, Devi LA. Expression of rat cathepsin S in phagocytic cells. *J Biol Chem*. 1996;271:4403–9.

335 Kingham PJ, Pocock JM. Microglial secreted cathepsin B induces neuronal apoptosis. *J Neurochem*. 2001;76:1475–84.

336 Wendt W, Schulten R, Stichel CC, Lübbert H. Intra-versus extracellular effects of microglia-derived cysteine proteases in a conditioned medium transfer model. *J Neurochem*. 2009;110:31–41.

337 Liu J, Hong Z, Ding J, Liu J, Zhang J, Chen S. Predominant release of lysosomal enzymes by newborn rat microglia after LPS treatment revealed by proteomic studies. *J Proteome Res*. 2008;7:2033–49.

338 Xu S, Zhang H, Yang X, Qian Y, Xiao Q. Inhibition of cathepsin L alleviates the microglia-mediated neuroinflammatory responses through caspase-8 and NF-kB pathways. *Neurobiol Aging*. 2018;62:159–67.

339 Yan B-Z, Chen L-Y, Kang L, Wang X-R, Bi M-R, Wang W, et al. Hepatoprotective effects of cathepsin B inhibitor on acute hepatic failure induced by lipopolysaccharide/D-galactosamine in mice. *Hepatobiliary Pancreat Dis Int*. 2013;12:80–6.

340 Fan K, Li D, Zhang Y, Han C, Liang J, Hou C, et al. The induction of neuronal death by up-regulated microglial cathepsin H in LPS-induced neuroinflammation. *J Neuroinflammation*. 2015;12:54.

341 Fan K, Wu X, Fan B, Li N, Lin Y, Yao Y, et al. Up-regulation of microglial cathepsin C expression and activity in lipopolysaccharide-induced neuroinflammation. *J Neuroinflammation*. 2012;9:96.

342 Pišlar A, Tratnjek L, Glavan G, Zidar N, Zivin M, Kos J. Neuroinflammation-induced upregulation of glial cathepsin X expression and activity in vivo. *Front Mol Neurosci*. 2020;13:1–17.

343 Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, et al. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat Immunol*. 2008;9:857–65.

344 Chen N, Ou Z, Zhang W, Zhu X, Li P, Gong J. Cathepsin B regulates non-canonical NLRP3 inflammasome pathway by modulating activation of caspase-11 in Kupffer cells. *Cell Prolif*. 2018;51:e12487.

345 Liang J, Li N, Zhang Y, Hou C, Yang X, Shimizu T, et al. Disinhibition of cathepsin C caused by catstatin F deficiency aggravates the demyelination in a cuprizone model. *Front Mol Neurosci*. 2016;9:152.

346 Liu Q, Zhang Y, Liu S, Liu Y, Yang X, Liu G, et al. Cathepsin C promotes microglia M1 polarization and aggravates neuroinflammation via activation of Ca2+-dependent PKC/p38MAPK/NF-kB pathway. *J Neuroinflammation*. 2019;16:10.

347 Hayashi Y, Koyanagi S, Kusunose N, Okada R, Wu Z, Tozaki-Saitoh H, et al. The intrinsic microglial molecular clock controls synaptic strength via the circadian expression of cathepsin S. *Sci Rep*. 2013;3:2744.

348 Glanzer JG, Enose Y, Wang T, Kadiu I, Gong N, Rozek W, et al. Genomic and proteomic microglial profiling: pathways for neuroprotective inflammatory responses following nerve fragment clearance and activation. *J Neurochem*. 2007;102:627–45.

349 Pišlar A, Božič B, Zidar N, Kos J. Inhibition of cathepsin X reduces the strength of microglial-mediated neuroinflammation. *Neuropharmacology*. 2017;114:88–100.

350 Greco TM, Seeholzer SH, Mak A, Spruce L, Ischiopoulos H. Quantitative mass spectrometry-based proteomics reveals the dynamic range of primary mouse astrocyte protein secretion. *J Proteome Res*. 2010;9:2764–74.

351 Stichel CC, Luebbert H. Inflammatory processes in the aging mouse brain: participation of dendritic cells and T-cells. *Neurobiol Aging*. 2007;28:1507–21.

352 Allan ERO, Campden RI, Ewanchuk BW, Taylor P, Balce DR, McKenna NT, et al. A role for cathepsin Z in neuroinflammation provides mechanistic support for
an epigenetic risk factor in multiple sclerosis. *J Neuroinflammation*. 2017;14:103.

353 Huynh JL, Garg P, Thin TH, Yoo S, Dutta R, Trapp BD, et al. Epigenome-wide differences in pathology-free regions of multiple sclerosis-affected brains. *Nat Neurosci*. 2014;17:121–30.

354 Haves-Zburof D, Paperna T, Gour-Lavie A, Mandel I, Glass-Marmor L, Miller A. Cathepsins and their endogenous inhibitors cystatins: expression and modulation in multiple sclerosis. *J Cell Mol Med*. 2011;15:2421–9.

355 Nagai A, Murakawa Y, Terashima M, Shimode K, Umegae N, Takeuchi H, et al. Cystatin C and cathepsin B in CSF from patients with inflammatory neurologic diseases. *Neurology*. 2000;55:1828–32.

356 Bever CT, Panitch HS, Johnson KP. Increased cathepsin B activity in peripheral blood mononuclear cells of multiple sclerosis patients. *Neurology*. 1994;44:745–8.

357 Bever CT, Garver DW. Increased cathepsin B activity in multiple sclerosis brain. *J Neurol Sci*. 1995;131:71–3.

358 Clark AK, Makkangio M. Microglial signalling mechanisms: cathepsin S and fractalkine. *Exp Neurol*. 2012;234:283–92.

359 Vrethem M, Kvarnström M, Stenstam J, Cassel P, Gustafsson M, Landtblom AM, et al. Cytokine mapping in cerebrospinal fluid and blood in multiple sclerosis patients without oligoclonal bands. *Mult Scler*. 2012;18:669–73.

360 Jagesaar SA, Holtman IR, Hofman S, Morandi E, Heijmans N, Laman JD, et al. Lymphocryptovirus infection of nonhuman primate B cells converts destructive into productive processing of the pathogenic CD8 T cell epitope in myelin oligodendrocyte glycoprotein. *J Immunol*. 2016;197:1074–88.

361 Schultz ML, Tecedor L, Chang M, Davidson BL. Clarifying lysosomal storage diseases. *Trends Neurosci*. 2011;34:401–10.

362 De Pasquale V, Moles A, Pavone LM. Cathepsins in the pathophysiology of mucopolysaccharidoses: new perspectives for therapy. *Cells*. 2020;9:979.

363 Steinfeld R, Reinhardt K, Schreiber K, Hillebrand M, Kraetzner R, Bruck W, et al. Cathepsin D deficiency is associated with a human neurodegenerative disorder. *Am J Hum Genet*. 2006;78:988–98.

364 Tang C-H, Lee J-W, Galvez MG, Robillard L, Mole SE, Chapman HA. Murine cathepsin F deficiency causes neuronal lipofuscinosis and late-onset neurological disease. *Mol Cell Biol*. 2006;26:2309–16.

365 Ketterer S, Gomez-Auli A, Hillebrand LE, Petrera A, Ketscher A, Reinheckel T. Inherited diseases caused by mutations in cathepsin protease genes. *FEBS J*. 2017;284:1437–54.

366 Smith KR, Dahl H-HM, Canafoglia L, Andermann E, Damiano J, Morbin M, et al. Cathepsin F mutations cause Type B Kufs disease, an adult-onset neuronal ceroid lipofuscinosis. *Hum Mol Genet*. 2013;22:1417–23.

367 Di Fabio R, Moro F, Pestillo L, Meschini MC, Pezzini F, Doccini S, et al. Pseudo-dominant inheritance of a novel CTSF mutation associated with type B Kufs disease. *Neurology*. 2014;83:1769–70.

368 van der Zee J, Mariën P, Crols R, Van Moorselde S, Dillen L, Perrone F, et al. Mutated CTSF in adult-onset neuronal ceroid lipofuscinosis and FTD. *Neuronal Genet*. 2016;2:e102.

369 Wang C, Xu H, Yuan Y, Lian Y, Xie N, Ming L. Novel compound heterozygous mutations causing Kufs disease type B. *Int J Neurosci*. 2018;128:573–6.

370 Koike M, Nakanishi H, Saftig P, Ezaki J, Ishihara K, Ohsawa Y, et al. Cathepsin D deficiency induces lysosomal storage with ceroid lipofuscin in mouse CNS neurons. *J Neurosci*. 2000;20:6898–906.

371 Amritraj A, Peake K, Kodam A, Salio C, Merighi A, Vance JE, et al. Increased activity and altered subcellular distribution of lysosomal enzymes determine neuronal vulnerability in Niemann-Pick type C1-deficient mice. *Am J Pathol*. 2009;175:2540–56.

372 Cermak S, Kosicek M, Mladenovic-Djordjevic A, Smiljanic K, Kanazir S, Hecimovic S. Loss of cathepsin B and L leads to lysosomal dysfunction, NPC-like cholesterol sequestration and accumulation of the key Alzheimer’s proteins. *PLoS One*. 2016;11:e0167428.

373 Zhu W, Shao Y, Yang M, Jia M, Peng Y. Asparaginyl endopeptidase promotes proliferation and invasiveness of prostate cancer cells via PI3K/AKT signaling pathway. *Gene*. 2016;594:176–82.