Review

Cathepsin D—Managing the Delicate Balance

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Abstract: Lysosomal proteases play a crucial role in maintaining cell homeostasis. Human cathepsin D manages protein turnover degrading misfolded and aggregated proteins and favors apoptosis in the case of proteostasis disruption. However, when cathepsin D regulation is affected, it can contribute to numerous disorders. The down-regulation of human cathepsin D is associated with neurodegenerative disorders, such as neuronal ceroid lipofuscinosis. On the other hand, its excessive levels outside lysosomes and the cell membrane lead to tumor growth, migration, invasion and angiogenesis. Therefore, targeting cathepsin D could provide significant diagnostic benefits and new avenues of therapy. Herein, we provide a brief overview of cathepsin D structure, function, and its role in the progression of many diseases and the therapeutic potentialities of natural and synthetic inhibitors and activators of this protease.

Keywords: cathepsin D; lysosome; regulated cell death; neurodegenerative disease; malignant tumor; diabetes; inhibitors

1. Introduction

Lysosomes are spherical acidic vesicles found in all mammalian cells but erythrocytes. Their primary role is the disposal and the recycling of exhausted and deteriorated macromolecules and organelles, along with the digestion of alien structures supplied by endocytosis [1]. Lysosomes contain more than 60 hydrolytic enzymes, including proteases, lipases, nucleases, glycosidases, phospholipases, phosphatases, and sulfatases [2]. Their acidic environment (pH 4–5), optimal for the activity of these enzymes, is maintained by a vacuolar-type H+-adenosine triphosphatase (ATPase) pumping protons from the cytosol into the lysosome lumen [3]. Cathepsins are the main mammalian lysosomal...
proteases, and they are classified into three groups according to their catalytic mechanism: Cysteine cathepsins (B, C, F, H, K, L, O, S, V, W, and X), serine cathepsins (A and G), and aspartic cathepsins (D and E) [4]. Most of them possess endopeptidase activity (cleaving internal peptide bonds), whereas some possess exopeptidase activity (cleaving off amino acid residues at the N- or C-terminal domains). Some cathepsins are both endopeptidases and exopeptidases, and their activity depends on their localization and environmental pH. There are many levels of cathepsin activity regulation, including biosynthetic processes, trafficking to lysosomes and other compartments, (auto-)proteolytic activation cleavage, and endogenous inhibitors [5–8]. They take part in tissue and bone remodeling, major histocompatibility complex class II-mediated antigen processing and presentation, protein, hormone, and neuropeptide processing, wound healing, apoptosis, as well as in disease development and progression, including cancer, inflammation, atherosclerosis, rheumatoid arthritis and neurodegeneration [9–13].

The aspartic protease cathepsin D is the most abundant lysosomal protease [14]. The human gene (CTSD) is located at the 11p15.5 region and contains 9 exons [15]. A mixed promoter controls cathepsin D gene expression, enabling both TATA-independent (binding site for specificity protein (Sp) 1) and TATA-dependent transcription initiations. Also, it was reported that its transcription might be activated by estrogen [16]. The encoded human cathepsin D protein comprises 412 amino acid residues. Following the removal of the signal peptide and two Asn glycosylations (134 and 263pre-pro-cathepsin D numbering) in the endoplasmic reticulum, the propeptide is transported to the endolysosomal compartment via mannose-6-phosphate (M6P)-dependent or independent (via low-density lipoprotein receptor (LDL-R) or LDL-R-related protein 1 (LRP1) receptor) pathways [17]. Once it reaches the lysosomes, the 52 kDa human pro-cathepsin D is proteolytically processed to the 48 kDa intermediate form that is further processed into the mature two-chain enzymatic form (a heavy 34 kDa chain and a light 14 kDa chain) [18]. This final maturation step occurs via cathepsins B and L activity [19] (Figure 1a). There is evidence that progranulin promotes in vitro maturation of pro-cathepsin D in a concentration-dependent fashion [20]. It was proposed that progranulin binds to the propeptide, destabilizing its interaction with the catalytic center of cathepsin D and promoting its autocatalytic activation cleavage [20]. Multi-granulin domain peptides (progranulin cleaved into smaller domains) showed an even more significant effect on the cathepsin D maturation, in a concentration- and pH-dependent manner [21]. The mature cathepsin D consists of two domains flanking the deep active site cleft [22]. Each domain provides a catalytic Asp to the catalytic site (Figure 1b). The two Asp active site residues are prone to deprotonation, suggesting that cathepsin D is predominantly active at pH below 5, as found in lysosomes. Nevertheless, recent data indicate that cathepsin D can also be active at higher pH in extravascular space and the cytoplasm [23–25].

Cathepsin D has endopeptidase activity and is responsible for the degradation of misfolded, long-lived and denatured proteins, such as the acid-denatured cathepsin L [26,27]. Moreover, it modulates the activity of diverse polypeptides, enzymes, and growth factors, and is thereby an essential regulator of cell signaling [28], while cathepsin D imbalance might play an important role in acute kidney injury [29], Huntington’s disease [30], Parkinson’s disease [31], Alzheimer’s disease [32], pancreatitis [33] and coronary events [34,35], making cathepsin D a vital protease for maintaining cellular homeostasis.
2. Vital Functions of Cathepsin D

Cathepsin D is ubiquitously expressed in various tissues where it is involved in protein turnover. Higher levels of cathepsin D expression in the brain, including the cortex, hippocampus, striatum, and dopaminergic neurons of the substantia nigra also suggest its essential role in the proteolysis of many altered neuronal proteins [37,38]. Long-lived post-mitotic cells, like neurons, depend on cellular homeostasis, which they preserve by an efficient proteostasis system regulating protein synthesis, folding and degradation balance [31]. Cellular health depends on the removal of damaged biological molecules, as well as on the turnover of organelles with a short lifespan. These phenomena can occur via autophagosome generation where endoplasmic reticulum membranes cloak and sequester the organelles and other intracellular materials to remove [39]. A process known as macroautophagy begins when the primary lysosome is merged with the autophagosome. In this scenario, cathepsin D deficiency has been identified as one of the causes of severe autophagy blockage in mice [40].

Cathepsin D is also necessary for proteostasis recovery after oxygen deprivation [41]. An increased protein aggregation characterizes the trophoblasts derived from patients with preeclampsia. Hypoxia-induced endoplasmic reticulum stress, one of the reasons for preeclampsia, can negatively affect transcription factor EB (TFEB) expression and its nuclear translocation leading to the decreased expression of TFEB-regulated lysosomal proteins, like lysosomal-associated membrane protein 1 (LAMP1), LAMP2, and cathepsin D. Impaired lysosomal biogenesis and autophagy resulted in increased protein aggregate accumulation in the placenta, due to the lack of lysosomal components [41]. In neurons, a low lysosomal enzymatic turnover, including cathepsin D, affects cell recovery following ischemic stroke even after mTOR activation induced by oxygen-glucose deprivation/reoxygenation [42]. In contrast, in a murine model, neuronal cathepsin D expression was shown to protect the brain against stroke injury by improving the lysosomal function [43]. Cathepsin D and cathepsin B double-knockout in mice was shown to cause impaired autophagy in the pancreas, which induced chronic pancreatitis [44]. Cathepsin D also takes part in autophagy during atherosclerosis [45].

In vitro experiments demonstrated a cathepsin D increase during apoptosis and other mechanisms of regulated cell death [46]. In this scenario, the bis-aryl urea derivative N69B demonstrated its anticancer activity by increasing cathepsin D-mediated tumor cell apoptosis through the B-cell lymphoma 2 (Bcl-2) homology domain 3 interacting domain-death agonist (Bid)/Bcl-2-like protein 4 (Bax)/cytochrome C/caspase 9/caspase 3 pathway.

Figure 1. Cathepsin D maturation and structure: (a) Major steps of cathepsin D maturation. The description is in the text. SP—signal peptide; Pro—prodomain; kDa—kilodaltons; ER—endoplasmic reticulum. (b) Mature cathepsin D structure. The two domains are indicated in different shades of green. Catalytic Asp are represented as sticks. The structure was obtained from Protein Data Bank (PDB ID: 6QCB) [36]. The figure was made in the PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.
Another study performed on the human neuroblastoma SH-SY5Y cell line demonstrated the cathepsin D involvement in the mitophagy process [48]. Namely, it was reported that mitophagy induction leads to enhanced expression of TFEB, which in turn, increases the synthesis of lysosomal proteins, including cathepsin D.

Cathepsin D is also involved in placenta antibacterial and antiviral defense when expressed with Napsin A [49]. They process the hemoglobin subunit β (HBB) to generate the bioactive peptide HBB\textsubscript{112–147}. Moreover, cathepsin D takes part in epidermal differentiation and barrier repair [50], damaged mitochondria degradation [51], promotes the fibrogenic potential in hepatic stellate cells [52] and favors cathepsin B activation [33]. It was suggested that cathepsin D might play an essential role in central nervous system (CNS) myelination since it is involved in cholesterol and lipid metabolism [53,54]. Recently, it was shown that zymogen form of cathepsin D possesses phosphatase activity in the cytosolic compartment [55]. The protease dephosphorylates cofilin, which in turn modulates actin remodelling and cell mitosis.

Despite the multiple roles of cathepsin D, the involvement of this protease in CNS proteostasis is the most investigated area. The importance of cathepsin D for the normal functioning of CNS is emphasized by many neurodegenerative diseases caused by cathepsin D deprivation. Cathepsin D deficiency resulted in fatal neurological disorders characterized by a significant loss of neurons and myelin in human infants and sheep [56], as well as in cell death in several tissues, including the brain [57], intestine, lymphoid organs [58] and the retina [59].

3. Cathepsin D Down-Regulation in Neurodegenerative Diseases

Reduced proteolytic activity of lysosomal cathepsins (including cathepsin D) was shown to induce a progressive accumulation of undigested autophagic substrates and unfolded or oxidized protein aggregates within the lysosomes during aging. This phenomenon can cause lysosomal membrane damage, culminating in cathepsin leakage and consequent apoptosis, favoring neurodegeneration [60].

Most neurodegenerative conditions can be distinguished in idiopathic (derived from unknown causes) and familial forms (linked to the gene mutation) [61]. Familial forms are characterized by continuous aggregation of short or immature proteins. Many mutated neuronal proteins such as amyloid precursor, α-synuclein, and huntingtin are cathepsin D substrates [32,60]. Some experiments demonstrated the cathepsin D involvement in the metabolism of cholesterol and glycosaminoglycans determining neuronal plasticity. Mutations in the CTSD gene are associated with different neurological defects [62]. CTSD gene homozygous inactivation was reported to cause human congenital neuronal ceroid lipofuscinosis (NCL) with postnatal respiratory insufficiency, epilepsy, and death within hours to weeks after birth, due to neurological defects and absence of neuronal α-synuclein accumulation [56]. Significant loss of cathepsin D enzymatic function due to the CTSD gene heterozygous missense mutations is associated with childhood motor and visual disturbances, cerebral and cerebellar atrophy, as well as progressive psychomotor disability. NCL3, a group of rare recessive lysosomal storage diseases, is associated with mutations in 14 different genes, including CTSD [63]. The main NCL3 symptoms are early blindness and severe progressive neurodegeneration. The missense variant in ceroid lipofuscinosis 5 (CLN5) c.A959G (p.Asn320Ser) correlates with Alzheimer’s disease occurrence [64]. More precisely, the CLN5 c.A959G variant causes a defect in the transport of cathepsin D to the endolysosomal compartment, resulting in an increased pro-cathepsin D level and a reduced mature cathepsin D expression [64]. Mutations and polymorphisms of cathepsin D linked with the NCL are summarized in Table 1.
Table 1. Cathepsin D mutations related to neuronal ceroid lipofuscinosis (NCL).

| DNA Change | Type of Mutation | Effect on Protease | NCL Type | Ref. |
|------------|------------------|-------------------|----------|-----|
| c.392A>G/p.Tyr131Cys | Missense | Reduced enzymatic activity | Late infantile NCL (LINCL) | [65] |
| c.446G>T/p.Gly149Val | Missense | Reduced enzymatic activity | Juvenile NCL (JNCL) | [66] |
| c.1196G>A/p.Arg399His | Missense | Reduced enzymatic activity | Congenital CLN 2 (CLN10) | [67] |
| c.299C>T/p.Ser100Phe | Missense | Reduced enzymatic activity | | |
| c.764dupA/p.Tyr255 | Nonsense | Absence of protein | | |
| c.6517T>A/p.Phe229Ile | Missense | Reduced protein amount and enzymatic activity | NCL-like disorder | [63] |
| c.10267G>C/p.Trp383Cys | | | | |

1 deoxyribonucleic acid; 2 ceroid-lipofuscinosis, neuronal.

In line with these data, any misbalance in protein degradation processes, such as autophagy and lysosomal hydrolytic enzyme activity is linked to some age-associated neurodegenerative disorders (e.g., Parkinson’s, Alzheimer’s, Huntington’s, and Niemann-Pick disease type C (NPC)) [31]. NPC is portrayed with intracellular accumulation of lipids, among which there are mainly cholesterol and glycosphingolipids. One of the tissues affected by this disease is the brain tissue [68]. Vomeronasal neuroepithelium in an NPC1 mouse model showed virtually absence of cathepsin D reactivity [69].

Some forms of cathepsin D deficiency could also predispose to late-onset Alzheimer’s disease and Parkinson’s disease [32]. The polymorphism c.C224T (p.Ala58Val) located in exon 2 of the CTSD gene was associated with sporadic late-onset Alzheimer’s disease in the adult German, Iranian, and Ecuadorian populations [70]. However, in other adult populations, e.g., Spanish, Italian, Korean, and North American, this association could not be established [70]. Alzheimer’s disease histopathology is characterized by accumulations of hyperphosphorylated $\tau$ protein and amyloid $\beta$ (A$\beta$). Mouse models of Alzheimer’s disease showed that A$\beta$ aggregates increase astrocyte lysosome pH, resulting in a decreased cathepsin D activity and lysosome damage [71]. In support of this evidence, extralysosomal cathepsin D was detected via confocal microscopy after long-term exposure of microglia to A$\beta$ aggregates following lysosomal membrane permeabilization [72]. Another mechanism was proposed by Suire et al. where A$\beta$42 inhibits cathepsin D in a low-nanomolar range, and thus, prevents cathepsin D degradation of $\tau$ protein [73]. Statistical analysis showed that cathepsin D level in plasma of patients with Alzheimer’s disease is decreased comparing to the subjects without cognitive impairment [74]. Therefore, cathepsin D can be used as a diagnostic biomarker for Alzheimer’s disease.

The unique role of cathepsin D in $\alpha$-synuclein proteolysis, which accumulates in Parkinson’s disease, has been proven. Sulfated glycosaminoglycans, which hoard in substantia nigra of Parkinson’s disease patients along with $\alpha$-synuclein, decrease the activity of cathepsin D [75]. The deficiency of glycosaminoglycans results in higher levels of proteolysis mediated by cathepsin D and consequent drop of $\alpha$-synuclein aggregates [75]. Earlier studies demonstrated that in vitro cathepsin D yields incomplete proteolysis of $\alpha$-synuclein and generates truncated C-terminal peptides, and in the acidic lysosomal lumen, enhance amyloid formation [76]. Later on, liquid chromatography-mass spectrometry analysis showed that anionic phospholipids are crucial for cathepsin D cleavage throughout $\alpha$-synuclein sequence [77]. Aufschnaiter et al. showed that cathepsin D proteolysis of $\alpha$-synuclein also needs calcineurin basal level expression [78].

Mutations in the $\beta$-Glucocerebrosidase gene (GBA1) are associated with Parkinson’s and Gaucher’s disease. Experiments on dopaminergic neurons and astrocytes carrying this mutation indicated excessive levels of $\alpha$-synuclein released from neurons that are eventually endocytosed by astrocytes [79]. $\alpha$-Synuclein accumulated in the lysosomes where it aggregated due to reduced cathepsin D activity. Other experiments also established decreased cathepsin D activity in substantia nigra and frontal cortex of patients with
Parkinson’s disease and Lewy body dementia [80]. Conversely, some studies showed increased levels of cathepsin D in Parkinson’s fibroblasts [81] and dopaminergic neurons [82]. In this context, Puska et al. showed that cathepsin D levels are increased while α-synuclein forms pre-aggregates [83]. However, the formation of Lewy bodies decreases the cathepsin D level. A possible explanation for cathepsin D low levels in patients with Parkinson’s disease might be the down-regulation of the M6P receptor due to its compromised retrograde transport from endosomes to the trans-Golgi network [84]. The study conducted in an ATP13A2 deficient zebrafish (Parkinson’s disease model) confirmed the reduced cathepsin D expression and showed lysosomal abnormalities, leading to the degeneration of dopaminergic neurons arguably caused by intracellular trafficking impairment [85]. Decreased levels of cathepsin D were also detected in plasma of patients with Parkinson’s disease comparing to the patients with essential tremor [86]. Therefore, all of the above data qualify cathepsin D as one of the biomarkers of Parkinson’s disease [87,88].

The possible role of cathepsin D in the development of prion diseases was demonstrated in interferon-α/β receptor knock-out mice showing decreased levels of disease-associated microglial cathepsin D and CD68 receptor, especially in white matter, which resulted in the slow disease progression [89].

Therefore, the deficiency of cathepsin D triggered by misregulation of its transport, maturation, and enzymatic activity results in the anomalous deposit of undigested cellular material in lysosomes [60]. Accumulations in lysosomes during aging weaken the lysosomal membrane causing enzymatic leakage, cell death and neurodegenerative disorders [90]. Everything aforesaid leads to the conviction that neuronal cellular health largely relies on cathepsin D-mediated proteolysis [60]. Therefore, the perspective approach in preventing the onset of a neurodegenerative disorder is to maintain a sufficient level of cathepsin D within the lysosomes.

4. Treatment to Restore Cathepsin D

Cathepsin D deficiency caused by mutations in the CTSD gene can be restored via recombinant protease as proposed by Marques et al. for NCL replacement therapy [40]. Specifically, the protocol was based on recombinant human pro-cathepsin D synthesized and purified from the human HEK 293 EBNA kidney cell line and delivered to the lysosomes, where it could be processed to its active form digesting protein aggregates. Possible difficulties to this approach were represented by non-functional M6P and LRP1 receptors and the cysteine protease activity [40].

In the case of a high level of pro-cathepsin D and a low level of the active form of the enzyme, cathepsin D maturation can be induced. As aforementioned, there is evidence that progranulin promotes in vitro maturation of pro-cathepsin D in a concentration-related process [20]. The maturation of pro-cathepsin D to its active form is stimulated even more significantly in the presence of multi-granulin domain peptides BAC and CDE resulting in an 80% active cathepsin D [21]. This might explain the reason why the progranulin gene therapy improves lysosomal dysfunction and microglial pathology associated with frontotemporal dementia and NCL [91].

Some neurodegenerative diseases are associated with a low cathepsin D level expression or activity inhibition. Eight lysosomotropic drugs (chloroquine, fluoxetine, imipramine, latrepirdine, tamoxifen, chlorpromazine, amitriptyline, and verapamil) were shown to increase cathepsin D activity at multiple concentrations after 24-h exposure [92]. A higher cathepsin D level was recorded within 4 h of latrepirdine and chlorpromazine treatment. A proteomic study in an in vivo model of depression showed that fluoxetine administration strongly up-regulated the expression of cathepsin D, proteins engaged in the improvement of learning and memory processes (stathmin 1 and dynamin-1), and proteins involved in mitochondrial biogenesis and defense against oxidative stress (protein deglycase DJ-1) [93]. *Mycobacterium tuberculosis* can inhibit phagosome maturation in infected macrophages by reducing galectin-3 expression [94]. This phenomenon can affect the development of the active cathepsin D. However, galectin-3 and cathepsin D expression could
be restored by treatment with gallium encapsulated in polymeric nanoparticles favoring infection inhibition [94]. Experiments on a mouse model of Alzheimer’s disease produced evidence that cilostazol can reinstate low pH in astrocyte lysosomes, consequently suspending the inhibitory effect of Aβ on the activity of cathepsin D [71]. Chlorogenic acid is another compound proven to have a neuroprotective role in an Alzheimer’s disease mouse model [95]. Specifically, chlorogenic acid was shown to up-regulate cathepsin D and other cathepsins expression via the mTOR/TFEB signaling pathway in APP/PS1 mice (Alzheimer’s disease model) and Aβ_{25-35}-exposed SH-SY5Y cells [95]. It was shown that glucocerebrosidase replacement or chaperone therapy of GBA1-mutant resulted in restored cathepsin D protein levels and activity, leading to decreased levels of monomeric α-synuclein in GBA1-mutant neurons [96].

There are multiple approaches to increase active cathepsin D in the cells. However, it is necessary to understand the kind of mechanism that caused the protease deprivation at first. There are also some risks associated with the modulation of this fragile balance that could favor the onset of other disorders.

5. Excessive Levels of Cathepsin D in Neurodegenerative Disorders

As mentioned earlier, Aβ aggregates can decrease the cathepsin D activity within the lysosomes. However, high exosomal levels of cathepsin D and LAMP1 together with low levels of the 70 kDa heat shock proteins were detected in the blood of patients with Alzheimer’s disease, suggesting a diagnostic role for this protease [97]. In addition, it was shown that the levels of cathepsin D are similar among patients with mild and severe Alzheimer’s disease and mild cognitive impairment [98]. On the other hand, the distinction between these groups of patients can be determined by comparing cathepsin B and cathepsin S levels [98]. Although, these results are inconsistent with the above-mentioned ones, so further research is required [74].

Glutaric acidemia type I (GA1) is a chronic progressive neurodegeneration caused by severe deficiency of glutaryl-CoA dehydrogenase activity, leading to glutaric acid and glutaryl carnitine accumulation [99]. It was demonstrated that brain-derived neurotrophic factor and cathepsin D significantly increased in the plasma of GA1 patients compared to the control group. Also, a positive correlation was found between the levels of cathepsin D and glutaryl carnitine levels that reflected the accumulation of glutaric acid. These data support the theory that glutaric acid is a critical player in the occurrence of neurological damage in GA1 patients [99].

The plasma of maple syrup urine disease patients showed increased levels of cathepsin D compared to the control group [100]. The authors proposed that high levels of cathepsin D may result from its role in cytokines- and oxidative stress-induced apoptosis. Increased cathepsin D levels were also observed in neurofibrillary tangles of parietal cortex neurons, where it correlated with hyperphosphorylated τ protein, but did not co-localize with α-synuclein inclusions [101].

Most neurodegenerative disorders caused by cathepsin D imbalance are characterized by down-regulation of the protease. However, there is evidence that in pathological conditions it can increase extracellularly, while the lysosomal concentration of cathepsin D can decrease.

6. Excessive Levels of Cathepsin D in Disorders Associated with Diabetes

Hyperglycemia can trigger cathepsin D release from the lysosomes by inducing lysosomal membrane permeabilization and ion release. Cathepsin D can remain active in non-acidic pH environments such as the cytosol. Altogether, this contributes to hyperglycemia-induced cardiomyocyte injury in patients with diabetic cardiomyopathy [102]. These results correlate with Hoef et al.’s study, which concluded that higher circulating cathepsin D levels correlate with greater heart failure severity [103]. Also, Liu et al. demonstrated a correlation between increased cathepsin D levels and type 2 diabetes [104].
The data concerning the influence of excessive cathepsin D activity outside the lysosomes on the severity of diabetes are relatively scarce. Further investigation of the mechanisms of cathepsin D regulation in this disorder may reveal new approaches for anti-diabetic therapy. Nevertheless, accumulated data detail the connection between lysosomal protease release and a pathological condition.

7. Excessive Levels of Cathepsin D in Malignant Tumors

Increased extracellular levels of cathepsins are well-characterized for tumors, and cathepsin D is no exception. High levels of cathepsin D were observed in breast [105], ovarian [106], colorectal [51], prostate, bladder cancer [107] and melanoma [108]. Numerous studies found that cathepsin D level may represent an independent prognostic factor in many cancers and is considered a potential target of anticancer therapy [109]. Cathepsin D was shown to have pro-angiogenesis, pro-apoptotic, pro-invasive and pro-metastatic properties [28]. Some research suggests the roles of cathepsin D in cancer cells in maintaining lysosomal integrity, redox balance and nuclear factor erythroid 2-related factor 2 activity, thus, promoting tumorigenesis [110]. There is evidence that even mutated cathepsin D deprived of catalytic activity can still have mitogenic properties by activating an unknown cell surface receptor [111].

Progesterone receptor isoforms A and B (PR-A and PR-B) ratio is used as a prognostic factor in breast cancer [112]. Breast cancer cells expressing PR-A show increased levels of proteins involved in the citric acid cycle, glycolysis, the Rho family of guanosine triphosphatase signaling, and ribonucleic acid (RNA) metabolism. Pateetin et al. showed an increased cathepsin D level in cells expressing progesterone-ligated PR-A [113]. Cathepsin D is secreted in estrogen-dependent and estrogen-independent types of breast cancer [114,115], and for this reason, represents a significant prognostic marker. Estradiol-mediated enhanced secretion of pro-cathepsin D in breast cancer cells was also established [116]. The suggested mechanism supports the involvement of the cation-dependent M6P receptor, which ensures the proper localization of the enzyme to lysosomes in MCF-7 cells (breast cancer) [116].

Liu et al. showed that tetrabromobisphenol A (TBBPA) could increase the extracellular and decrease the intracellular levels of cathepsins D and B in hepatocellular carcinoma (HCC) cell line, HepG2 [117]. These results imply that TBBPA might promote lysosomal exocytosis and consequent in vitro HepG2 cell invasion and metastasis. It is believed that TBBPA could bind mucolipin-1, forming a complex which significantly increases Ca^{2+}-mediated lysosomal exocytosis in HCC [117]. Increased lysosomal membrane permeabilization in HCC could result from suppressed sulfatase 2, which leads to lysosome-associated protein transmembrane 4β inhibition whose expression depends on sulfatase 2 [118]. Detection of extralysosomal cathepsin D represents a sign of disrupted autophagy.

The outcome of several studies claims that cathepsin D, together with cathepsin B, plays essential roles in the production of angiotensin peptides in glioblastoma cells bypassing the renin-angiotensin system [119]. Basu et al. demonstrated that colorectal cancer cells with overexpression of immunoglobulin-like cell adhesion receptor L1 also showed increased extra- and intra-cellular levels of cathepsin D [120]. They suggested the essential role of cathepsin D in colorectal cancer progression. Cathepsin D might represent a therapeutic target for curing invasive colorectal cancer, given that it is only detected in invasive areas of the tumor [120].

The abundance of cathepsin D outside the lysosomes in malignant tumors makes it a convenient marker and a target for cancer treatment. Still, there must be targeted delivery of the inhibitors to prevent the onset of off-site disorders.

8. Cathepsin D Inhibitors

Most cathepsin are cysteine proteases so their inhibitors belong to the cystatin superfamily, including stefins, kininogens, thyropins, and serpins [5]. However, there are no known endogenous inhibitors for the aspartic protease cathepsin D in mammals [121].
Nonetheless, there are several natural inhibitors isolated from other species. The most known inhibitor of cathepsin D is pepstatin A from Actinomyces, an inhibitor of aspartic proteases [76]. Baldwin et al. conducted the thorough comparative analysis of the native protease and the protease in complex with pepstatin A (PDB ID: 1LYA, 1LYB) [122]. However, non-specific inhibition of aspartic proteases, including cathepsin D, may induce side effects (e.g., CNS), and may not be safe as therapeutics [123]. Therefore, novel studies examine new possible inhibitors using a 2/3-dimensional quantitative structure-activity relationship, representing a powerful tool for explaining the relationships between chemical structure and experimental observations [124]. These studies showed that oxymatrine from *Sophora flavescens* down-regulated the expression of cathepsin D, inhibiting high mobility group protein 1/toll-like receptor 4/nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, resulting in the suppression of microglia-mediated neuroinflammation in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-stimulated mouse model of Parkinson’s disease [125]. Fucoidan from brown algae inhibited the expression of cathepsin D and Bax in the murine dopaminergic nerve precursor cell line, MN9D (neuroblastoma), and indicated its prospective role as a neuroprotective agent in Parkinson’s disease treatment [126]. Flavonoid morin hydrate from *Maclura pomifera*, *Maclura tinctoria*, and *Psidium guajava* is claimed to be anti-oxidative and anti-inflammatory. Recently, it was shown to inhibit cathepsin D in kidneys of mice with chronic kidney damage [127]. Molecular docking revealed that morin hydrate interacts with cathepsin D active site via H-bonds and hydrophobic interactions.

Some experiments demonstrated that a small-molecule inhibitor of β-secretase 1, aminothiazole-based compound LY2811376, inhibits cathepsin D activity as well [128]. This pH-dependent suppression results from a salt bridge formation between the inhibitor aspartyl binding motif and Asp$_{33}$ in cathepsin D. Furthermore, a group of scientists using a graph convolutional neural network (CNN) indicated that cathepsin D is an off-target ligand of some β-secretase 1 inhibitors [129]. Another work unraveled the neuroprotective role of necrostatin-1 against oxidative stress-induced cell damage [130]. It demonstrated that necrostatin-1 induced cathepsin D inhibition in a cell line of differentiated human neuroblastoma RA-SH-SY5Y. Recently, pseudo-dipeptide binding motif of pepstatin A was used to design macrocyclic peptidomimetic inhibitors [36]. They were shown to inhibit cathepsin D in nanomolar and sub-nanomolar range without citotoxicity in contrast to pepstatin A (PDB ID: 6QBG, 6QBH, 6QCB). The high throughput screening (HTS) identified a series of acylguanidine inhibitors which interact with catalytic Asp via H-bonds and inhibit cathepsin D with nanomolar potency [131]. However, they were characterized as inhibitors with low microsomal stability and permeability so further optimization is required (PDB ID: 4OBZ, 4OC6, 4OD9). Further optimization of the compounds resulted in the inhibitor 24e with improved microsomal stability, as shown on human and mouse liver microsomes [132].

Several studies indicated the effectiveness of combination therapy with natural and synthetic inhibitors. For instance, co-treatment with praeruptorin C from *Peucedanum praeruptorum* and U0126 synergistically inhibited cathepsin D expression through the extracellular signal-regulated kinase 1/2 signaling pathway in human non-small cell lung cancer cells [133]. There is evidence that autophagy modulators chloroquine, KU-55933, and rapamycin from *Streptomyces hygroscopicus* combined with a recombinant analog of human milk protein lactaptin decreased cathepsin D activity with cytotoxic effects in MDA-MB-231 cell line (breast carcinoma) [134].

RNA-based compounds might represent another approach to inhibit cathepsin D. It was shown that cathepsin D knockdown via CTSD shRNA lentiviral vector transduction suppressed lipopolysaccharide-induced neuroinflammation by inhibiting NF-κB signaling pathway [135]. This phenomenon was obtained by regulating the nuclear factor NF-κB p65 subunit (p65) nuclear translocation both in MPTP-challenged mice and lipopolysaccharide-induced murine microglia, BV-2 cell line [135]. Phosphatidylinositol
3-phosphate (PtdIns(3)P) RNA aptamer binding to PtdIns(3)P inhibited autophagy by hampering lysosomal acidification, which resulted in reduced cathepsin D activity [136].

Although some studies have shown that cathepsin inhibitors may be used as metastasis suppressors [137], others suggested that suppression of catstins may contribute to the suppression of breast cancer [138], colorectal cancer [139] pancreatic ductal adenocarcinoma [140]. Therefore, one has to be careful in developing a treatment, in order to avoid side effects. Nevertheless, this should not be an issue since cathepsin D inhibitors are represented by synthetic and natural compounds including peptides and RNAs, providing an extensive portfolio of therapies depending on the mechanisms of up-regulation, drug delivery, as well as severity of the disease. Cathepsin D inhibitors are summarized in Table 2.

Table 2. Cathepsin D inhibitors in chronological order.

| Inhibitor | Natural Compounds | Mechanism | Ref. |
|-----------|-------------------|-----------|------|
| Pepstatin A from Actinomycetes | Non-competitive inhibitor | [141] |
| Cycloheximide from Streptomyces griseus | Protein synthesis inhibitor | [142] |
| The 22-kDa cathepsin D inhibitor protein of potatoes (PDI) from Solanum tuberosum | Reversible inhibitor | [143] |
| Equistatin from Actinia equina | Reversible inhibitor | [144] |
| Fucoidan from brown seaweds and algae | Down-regulator of the expression | [126] |
| Oxymatrine from Sophora flavescens | Down-regulator of the expression | [125] |
| Morin hydrate from Maclura pomifera, Maclura tinctoria, Psidium guajava | Reversible inhibitor | [127] |

| Inhibitor | Synthetic compounds | Mechanism | Ref. |
|-----------|---------------------|-----------|------|
| Dithiophosgene | Irreversible covalent inhibitor | [145] |
| 2,2-Dichloro-1,3-dithiacyclobutanone | Irreversible covalent inhibitor | [146] |
| Diazo compounds | Reversible inhibitor | [147] |
| Pro-Pro-Phe-Phe-Val-D-Leu | Reversible inhibitor | [148] |
| Cbz-Val-Val-(3S4S)-statine | Reversible inhibitor | [149] |
| Ibu-His-Pro-HCys-Sta-Leu-NH-\[\text{CH}_2\text{S}-\text{Acm}\] | Reversible inhibitor | [150] |
| Derivatives of 4-(morpholinylsulphonyl)-L-Phe-P2-(cyclohexyl)Ala psii[isostere]-P1'-P2' | Irreversible covalent inhibitor | [76] |
| Lentiviral shRNA constructs | RNA interference inhibitor | [131,132] |
| Acylguanidines | Reversible inhibitor | [128] |
| LY281376 | Reversible inhibitor | [136] |
| PtdIns(3)P RNA aptamer | Inhibitor of PtdIns(3)P | [36] |
| Macrocyclic inhibitors | Competitive inhibitor | [130] |
| Necrostatin-1 | Suppressor of activity | [133] |

| Inhibitor | Polytherapy | Mechanism | Ref. |
|-----------|-------------|-----------|------|
| RL2, with chloroquine, Ku55933, and rapamycin from Streptomyces hygroscopicus | Suppressor of activity | [134] |
| Praeruptorin C from Peucedanum praeruptorum and U0126 | Inhibitor through ERK1/2 signaling pathway | [133] |

1 phospatidylinositol 3-phosphate; 2 recombinant analog of human milk protein lactaptin; 3 extracellular signal-regulated kinase 1/2.

9. Conclusions

Cathepsin D is involved in autophagy, endocytosis, degradation of misfolded or mutated proteins, regulation of the activity of various polypeptides, enzymes and growth factors. Due to its numerous roles in metabolic processes, necessary for cell survival and death, it is crucial to maintain the level of cathepsin D activity within optimal limits.

Decreased cathepsin D activity and/or levels have been observed in several neurodegenerative disorders (Figure 2). Replacement therapy with recombinant pro-cathepsin D seems a perspective approach to mitigate its reduced expression [40]. When it comes
to compensating cathepsin D reduced activity due to lysosome membrane permeability increase, the therapy should aim to maintain membrane homeostasis, preserving the pH and cholesterol levels in these organelles [151]. It is also necessary to explore the signaling pathways that lead to a change in lysosome membrane proteins conformation to develop appropriate inhibitors.

Figure 2. Role of Cathepsin D in cancer and neurological disorders. Cathepsin D mutations may cause a decrease in its lysosomal traffic and activity, leading to protein aggregation in the organelles and neurodegenerative diseases development. Protein aggregation may cause lysosomal membrane permeabilization with the subsequent increase of cathepsin D outside the lysosomes. On the other hand, the excessive extracellular activity of cathepsin D can contribute to tumorigenesis.

Lysosome membrane permeabilization also leads to excessive cathepsin D activity in the cytoplasm and extracellular space. This contributes to malignant tumor growth, metastasis and angiogenesis (Figure 2). The inhibitors may decrease cathepsin D activity, but the research for new, specific inhibitors should continue. It is necessary to emphasize that one should be extremely careful in using inhibitors for therapeutic purposes. As previously mentioned, cathepsin D participates in many processes in the whole organism. Therefore, the systematic use of inhibitors should be avoided, and targeted therapy should be investigated hand in hand with the development of novel therapeutics [152].

There are still several functions of cathepsin D to investigate. For example, cathepsin D is involved in the development of disorders associated with diabetes or its involvement in redox response suggesting the potential use of this protease in anti-photoaging therapies [50]. Therefore, there is an abundance of pathways for exploring future research in consideration of the many and various roles of cathepsin D.
References

1. Johansson, A.C.; Appelqvist, H.; Nilsson, C.; Kågedal, K.; Roberg, K.; Öllinger, K. Regulation of apoptosis-associated lysosomal membrane permeabilization. *Apoptosis* 2010, 15, 527–540. [CrossRef]

2. Trivedi, P.C.; Bartlett, J.J.; Pulinilkunnil, T. Lysosomal Biology and Function: Modern View of Cellular Debris Bin. *Cells* 2020, 9, 1131. [CrossRef]

3. Ishida, Y.; Nayak, S.; Mindell, J.A.; Grabe, M. A model of lysosomal pH regulation. *J. Gen. Physiol.* 2013, 141, 705–720. [CrossRef]

4. Patel, S.; Homaei, A.; El-Seedi, H.R.; Akhtar, N. Cathepsins: Proteases that are vital for survival but can also be fatal. *Biomed. Pharmacother.* 2018, 105, 526–532. [CrossRef] [PubMed]

5. Novincec, M.; Lenarčič, B.; Turk, B. Cysteine cathepsin activity regulation by glycosaminoglycans. *Biomed Res. Int.* 2014, 2014, 309718. [CrossRef] [PubMed]

6. Soond, S.M.; Kozhevnikova, M.V.; Zamyatnin, A.A. “Patchiness” and basic cancer research: Unravelling the proteases. *Cell Cycle* 2019, 18, 1687–1701. [CrossRef] [PubMed]

7. Soond, S.M.; Kozhevnikova, M.V.; Frolova, A.S.; Savvateeva, L.V.; Plotnikov, E.Y.; Townsend, P.A.; Han, Y.-P.; Zamyatnin, A.A. Lost or Forgotten: The nuclear cathepsin protein isoforms in cancer. *Cancer Lett.* 2019, 462, 43–50. [CrossRef]

8. Petushkova, A.I.; Zamyatnin, A.A. Redox-Mediated Post-Translational Modifications of Proteolytic Enzymes and Their Role in Protease Functioning. *Biomolecules* 2020, 10, 650. [CrossRef] [PubMed]

9. Pišlar, A.; Kos, J. Cysteine cathepsins in neurological disorders. *Mol. Neurobiol.* 2014, 49, 1017–1030. [CrossRef] [PubMed]

10. Petushkova, A.I.; Savvateeva, L.V.; Korolev, D.O.; Zamyatnin, A.A. Cysteine Cathepsins: Potential Applications in Diagnostics and Therapy of Malignant Tumors. *Biochemistry* 2019, 84, 746–761. [CrossRef]

11. Rudzińska, M.; Parodi, A.; Soond, S.M.; Vinarov, A.Z.; Korolev, D.O.; Morozov, O.A.; Daglioglu, C.; Tutar, Y.; Zamyatnin, A.A. The Role of Cysteine Cathepsins in Cancer Progression and Drug Resistance. *Int. J. Mol. Sci.* 2019, 20, 3602. [CrossRef]

12. Soond, S.M.; Savvateeva, L.V.; Makarov, V.A.; Gorokhovets, N.V.; Townsend, P.A.; Zamyatnin, A.A. Making Connections: p53 and the Cathepsin Proteases as Co-Regulators of Cancer and Apoptosis. *Cancers* 2020, 12, 3476. [CrossRef]

13. Soond, S.M.; Kozhevnikova, M.V.; Townsend, P.A.; Zamyatnin, A.A. Integrative p53, micro-RNA and Cathepsin Protease Co-Regulatory Expression Networks in Cancer. *Cancers* 2020, 12, 3454. [CrossRef]

14. Oberle, C.; Huai, J.; Reinehekel, T.; Tacke, M.; Rassner, M.; Ekert, P.G.; Buellesbach, J.; Borner, C. Lysosomal membrane permeabilization and cathepsin release is a Bax/Bak-dependent, amplifying event of apoptosis in fibroblasts and monocyes. *Cell Death Differ.* 2010, 17, 1167–1178. [CrossRef]

15. Lehesjoki, A.-E.; Gardiner, M. Progressive Myoclonus Epilepsy. In *Jasper’s Basic Mechanisms of the Epilepsies*; Oxford University Press: Oxford, UK, 2012; pp. 878–886.

16. Cavailles, V.; Augereau, P.; Rochefort, H. Cathepsin D gene is controlled by a mixed promoter, and estrogens stimulate only TATA-dependent transcription in breast cancer cells. *Proc. Natl. Acad. Sci. USA* 1993, 90, 203–207. [CrossRef]

17. Markmann, S.; Thelen, M.; Corrils, K.; Schweizer, M.; Brocke-Ahmadinejad, N.; Willnow, T.; Heeren, J.; Gieselmann, V.; Braulke, T.; Kollmann, K. Lrp1/LDL Receptor Play Critical Roles in Mannose 6-Phosphate-Independent Lysosomal Enzyme Targeting. *Traffic* 2015, 16, 743–759. [CrossRef]

18. Zaidi, N.; Maurer, A.; Nieke, S.; Kalbacher, H. Cathepsin D: A cellular roadmap. *Biochem. Biophys. Res. Commun.* 2008, 376, 5–9. [CrossRef] [PubMed]

19. Laurent-Matha, V.; Deroçq, D.; Prébois, C.; Katunuma, N.; Liaudet-Coopman, E. Processing of human cathepsin D is independent of its catalytic function and auto-activation: Involvement of cathepsins L and B. *J. Biochem.* 2006, 139, 363–371. [CrossRef] [PubMed]

20. Butler, V.J.; Cortopassi, W.A.; Argouarch, A.R.; Ivory, S.L.; Craik, C.S.; Jacobson, M.P.; Kao, A.W. Progranulin Stimulates the In Vitro Maturation of Pro-Cathepsin D at Acidic pH. *J. Mol. Biol.* 2019, 431, 1038–1047. [CrossRef] [PubMed]

21. Butler, V.J.; Cortopassi, W.A.; Gururaj, S.; Wang, A.L.; Pierce, O.M.; Jacobson, M.P.; Kao, A.W. Multi-Granulin Domain Peptides Bind to Pro-Cathepsin D and Stimulate Its Enzymatic Activity More Effectively Than Progranulin in Vitro. *Biochemistry* 2019, 58, 2670–2674. [CrossRef]

22. Metcalf, P.; Fusek, M. Two crystal structures for cathepsin D: The lysosomal targeting signal and active site. *EMBO J.* 1993, 12, 1293–1302. [CrossRef]
46. Shibata, M.; Kanamori, S.; Isahara, K.; Ohsawa, Y.; Konishi, A.; Kametaka, S.; Watanabe, T.; Ebisu, S.; Ishido, K.; Kominami, E.; et al. Participation of cathepsins B and D in apoptosis of PC12 cells following serum deprivation. Biochem. Biophys. Res. Commun. 1998, 251, 199–203. [CrossRef] [PubMed]

47. Wu, J.; Huang, Y.; Xie, Q.; Zhang, J.; Zhan, Z. A novel bis-aryl urea compound inhibits tumor proliferation via cathepsin D-associated apoptosis. Anticancer Drugs 2020, 31, 500–506. [CrossRef] [PubMed]

48. Ivanovic, D.; Chau, K.Y.; Schapira, A.H.V.; Gegg, M.E. Mitochondrial and lysosomal biogenesis are activated following PINK1/parkin-mediated mitophagy. J. Neurochem. 2016, 136, 388–402. [CrossRef] [PubMed]

49. Groß, R.; Bauer, R.; Krüger, F.; Rücker-Braun, E.; Ollari, L.R.; Ständker, L.; Pfeising, N.; Rodriguez, A.A.; Conzelmann, C.; Gerbl, F.; et al. A Placenta Derived C-Terminal Fragment of β-Hemoglobin with Combined Antibacterial and Antiviral Activity. Front. Microbiol. 2020, 11, 508. [CrossRef]

50. Xu, X.; Zheng, Y.; Huang, Y.; Chen, J.; Gong, Z.; Li, Y.; Lu, C.; Lai, W.; Xu, Q. Cathepsin D contributes to the accumulation of advanced glycation end products during photoaging. J. Dermatol. Sci. 2018, 90, 263–275. [CrossRef]

51. Oliveira, C.S.F.; Pereira, H.; Alves, S.; Castro, L.; Baltazar, F.; Chaves, S.R.; Preto, A.; Côte-Real, M. Cathepsin D protects colorectal cancer cells from acetate-induced apoptosis through autophagy-independent degradation of damaged mitochondria. Cell Death Dis. 2015, 6, e1788. [CrossRef]

52. Moles, A.; Tarrats, N.; Fernández-Checa, J.C.; Mari, M. Cathepsins B and D drive hepatic stellate cell proliferation and promote their fibrogenic potential. Hepatology 2009, 49, 1297–1307. [CrossRef] [PubMed]

53. Guo, D.-Z.; Xiao, L.; Liu, Y.-J.; Shen, C.; Lou, H.-F.; Lv, Y.; Pan, S.-Y. Cathepsin D deficiency delays central nervous system myelination by inhibiting proteolipid protein trafficking from late endosome/lysosome to plasma membrane. Exp. Mol. Med. 2018, 50, e457. [CrossRef] [PubMed]

54. Mutka, A.L.; Haapanen, A.; Käkelä, R.; Lindfors, M.; Wright, A.K.; Inkinen, T.; Hermansson, M.; Rokka, A.; Corthals, G.; Jauhiainen, M.; et al. Urinary cathepsin D deficiency is associated with dysmyelination/myelin disruption and accumulation of cholesterol esters in the brain. J. Neurochem. 2010, 112, 193–203. [CrossRef] [PubMed]

55. Liu, Y.-J.; Zhang, T.; Chen, S.; Cheng, D.; Wu, C.; Wang, X.; Duan, D.; Zhu, L.; Lou, H.; Gong, Z.; et al. The noncanonical role of the protease cathepsin D as a coflin phosphatase. Cell Res. 2021, 1–13. [CrossRef]

56. Siintola, E.; Partanen, S.; Strömme, P.; Haapanen, A.; Haltia, M.; Maehlen, J.; Lehesjoki, A.E.; Tyynelä, J. Cathepsin D deficiency underlies congenital human neuronal ceroid-lipofuscinosis. Brain 2006, 129, 1438–1445. [CrossRef] [PubMed]

57. Tian, L.; Zhang, K.; Tian, Z.-Y.; Wang, T.; Shang, D.-S.; Li, B.; Liu, D.-X.; Fang, W.-G.; Wang, Z.-Y.; Chen, Y.-H. Decreased expression of cathepsin D in monocytes is related to the defective degradation of amyloid-β in Alzheimer’s disease. J. Alzheimers. Dis. 2014, 134, 42, 511–520. [CrossRef]

58. Saftig, P.; Hetman, M.; Schmalw, W.; Weber, K.; Heine, L.; Mössmann, H.; Köster, A.; Hess, B.; Evers, M.; von Figura, K. Mice with a Retromer Trafficking Defect. Mol. Cell. Biol. 2018, 38, 443–496. [CrossRef] [PubMed]

59. Fritchie, K.; Siintola, E.; Armado, D.; Leshejski, A.-E.; Marino, T.; Powell, C.; Tennison, M.; Booker, J.M.; Koch, S.; Partanen, S.; et al. Novel mutation and the first prenatal screening of cathepsin D deficiency (CLN10). Acta Neuropathol. 2009, 117, 201–208. [CrossRef]
69. Witt, M.; Thiemer, R.; Meyer, A.; Schmitt, O.; Wree, A. Main Olfactory and Vomeronasal Epithelium are Differently Affected in Niemann-Pick Disease Type C1. Int. J. Mol. Sci. 2018, 19, 3563. [CrossRef] [PubMed]

70. Ketttwig, M.; Ohlenbusch, A.; Jung, K.; Steinfeld, R.; Gartner, J. Cathepsin D Polymorphism C224T in Childhood-Onset Neurodegenerative Disorders: No Impact for Childhood Dementia. J. Pediatr. Genet. 2018, 7, 14–18.

71. Kim, H.N.; Seo, B.-R.; Kim, H.; Koh, J.-Y. Cilostazol restores autophagy flux in bafilomycin A1-treated, cultured cortical astrocytes through lysosomal reacidification: Roles of PKA, zinc and metallothionein 3. Sci. Rep. 2020, 10, 9175. [CrossRef] [PubMed]

72. Pomilio, C.; Gorjorj, R.M.; Riudavets, M.; Vinuesa, A.; Presa, J.; Gregosa, A.; Bentivegna, M.; Alaimo, A.; Alcon, S.P.; Sevlever, G.; et al. Microglial autophagy is impaired by prolonged exposure to β-amyloid peptides: Evidence from experimental models and Alzheimer’s disease patients. Gerosci 2020, 42, 613–632. [CrossRef]

73. Sutre, C.N.; Leisstring, M.A. Cathepsin D: A Candidate Link between Amyloid β-protein and Tauopathy in Alzheimer Disease. J. Exp. Neurol. 2021, 2, 10–15.

74. Kim, J.-W.; Jung, S.-Y.; Kim, Y.; Heo, H.; Hong, C.-H.; Seo, S.-W.; Choi, S.-H.; Son, S.-J.; Lee, S.; Chang, J. Identification of Cathepsin D as a Plasma Biomarker for Alzheimer’s Disease. Cells 2021, 10, 138. [CrossRef]

75. Lehri-Boufala, S.; Ouidja, M.O.; Barbier-Chassefiere, V.; Henault, E.; Raisman-Vozari, R.; Garrigue-Antar, L.; Papy-Garcia, D.; Morin, C. New roles of glycosaminoglycans in α-synuclein aggregation in a cellular model of Parkinson disease. PLoS ONE 2015, 10, e0116641. [CrossRef]

76. Sevlever, D.; Jiang, P.; Yen, S.H.C. Cathepsin D is the main lysosomal enzyme involved in the degradation of α-synuclein and generation of its carboxy-termnally truncated species. Biochemistry 2008, 47, 9678–9687. [CrossRef] [PubMed]

77. McGlinchey, R.P.; Lee, J.C. Cysteine cathepsins are essential in lysosomal degradation of α-synuclein. Proc. Natl. Acad. Sci. USA 2015, 112, 9322–9327. [CrossRef]

78. Aufschnaiter, A.; Habernig, L.; Kohler, V.; Diessl, J.; Carmona-Gutierrez, D.; Eisenberg, T.; Keller, W.; Bittner, S. The coordinated action of calcineurin and cathepsin D protects against α-synuclein toxicity. Front. Mol. Neurosci. 2017, 10, 207. [CrossRef]

79. Affaki, E.; Stubbelefield, B.K.; McGlinchey, R.P.; McMahon, B.; Ory, D.S.; Sidransky, E. A characterization of Gaucher iPS-derived astrocytes: Potential implications for Parkinson’s disease. Neurobiol. Dis. 2020, 134, 104647. [CrossRef] [PubMed]

80. Moors, T.E.; Pacotti, S.; Ingrassia, A.; Quadri, M.; Breedveld, G.; Tasegian, A.; Chiasserini, D.; Eusebi, P.; Duran-Pacheco, G.; Kremer, T.; et al. Characterization of Brain Lysosomal Activities in GBA-Related and Sporadic Parkinson’s Disease and Dementia with Lewy Bodies. Mol. Neurobiol. 2019, 56, 1344–1355. [CrossRef] [PubMed]

81. McNeill, A.; Magalhaes, J.; Shen, C.; Chau, K.Y.; Hughes, D.; Mehta, A.; Foltynie, T.; Cooper, J.M.; Abramov, A.Y.; Gegg, M.; et al. Ambroxol improves lysosomal biochemistry in glucocerebrosidase mutation-linked Parkinson disease cells. Brain 2014, 137, 1481–1495. [CrossRef] [PubMed]

82. Fernandes, H.J.R.; Hartfield, E.M.; Christian, H.C.; Emmanoulidou, E.; Zheng, Y.; Booth, H.; Bogetofte, H.; Lang, C.; Ryan, B.J.; Sardi, S.P.; et al. Mannose 6-Phosphate Receptor Is Reduced in α-Synuclein Overexpressing Models of Parkinsons Disease. Stem Cells 2016, 6, 342–356. [CrossRef]

83. Puska, G.; Lutz, M.I.; Molnar, K.; Regelsberger, G.; Ricken, G.; Birker, W.; Laszlo, L.; Kovacs, G.G. Lysosomal response in relation to α-synuclein pathology differs between Parkinson’s disease and multiple system atrophy. Neurobiol. Dis. 2018, 114, 140–152. [CrossRef] [PubMed]

84. Matrone, C.; Dzamko, N.; Madsen, P.; Nyegaard, M.; Pohlmann, R.; Søndergaard, R.V.; Lassen, L.B.; Andresen, T.L.; Halliday, G.M.; Jensen, P.H.; et al. Mannose 6-Phosphate Receptor Is Reduced in α-Synuclein Overexpressing Models of Parkinsons Disease. PLoS ONE 2016, 11, e0160501. [CrossRef] [PubMed]

85. Nyuzuki, H.; Ito, S.; Nagasaki, K.; Nitta, Y.; Matsui, N.; Saitoh, A.; Matsui, H. Degeneration of dopaminergic neurons and impaired intracellular trafficking in Atip132a2 deficient zebrafish. IBRO Rep. 2020, 9, 1–8. [CrossRef]

86. Kang, J.; Kim, J.W.; Hae, H.; Lee, J.; Park, K.Y.; Yoon, J.H.; Chang, J. Identification of BAG2 and Cathepsin D as Plasma Biomarkers for Parkinson’s Disease. Clin. Transl. Sci. 2021, 14, 606–616. [CrossRef]

87. Parnetti, L.; Balducci, C.; Pigni, F., de Carlo, C.; Fpeduzzi, M.; D’Amore, C.; Padiglioni, C.; Mastrocola, S.; Persichetti, E.; Pacioti, S.; et al. Cerebrospinal fluid β-glucocerebrosidase activity is reduced in Dementia with Lewy Bodies. Neurobiol. Dis. 2009, 34, 484–486. [CrossRef]

88. Xicoy, H.; Peñuelas, N.; Vila, M.; Laguna, A. Autophagic- and Lysosomal-Related Biomarkers for Parkinson’s Disease: Lights and Shadows. Cells 2019, 8, 1317. [CrossRef]

89. Nazmi, A.; Field, R.H.; Griffin, E.W.; Haugh, O.; Hennessy, E.; Cox, D.; Reis, R.; Tortorelli, L.; Murray, C.L.; Lopez-Rodriguez, A.B.; et al. Chronic neurodegeneration induces type I interferon synthesis via STING, shaping microglial phenotype and accelerating disease progression. Glia 2019, 67, 1254–1276. [CrossRef] [PubMed]

90. Wu, F.; Xu, H.-D.; Guan, J.-J.; Hou, Y.-S.; Gu, J.-H.; Zhen, X.-C.; Qin, Z.-H. Rotenone impairs autophagic flux and lysosomal functions in Parkinson’s disease. Neuroscience 2015, 284, 900–911. [CrossRef]

91. Arrant, A.E.; Onyilo, V.C.; Unger, D.E.; Roberson, E.D. Progranulin gene therapy improves lysosomal dysfunction and microglial pathology associated with frontotemporal dementia and neuronal ceroid lipofuscinosis. J. Neurosci. 2018, 38, 2341–2358. [CrossRef] [PubMed]

92. Lu, S.; Sung, T.; Lin, N.; Abraham, R.T.; Jessen, B.A. Lysosomal adaptation: How cells respond to lysosomotropic compounds. PLoS ONE 2017, 12, e0173771. [CrossRef]
118. Ha, Y.; Fang, Y.; Romecin Duran, P.A.; Tolosa, E.J.; Moser, C.D.; Fernandez-Zapico, M.E.; Roberts, L.R. Induction of Lysosome-associated Protein Transmembrane 4 Beta via Sulphatase 2 Enhances Autophagic Flux in Liver Cancer Cells. *Hepatol. Commun.* 2019, 3, 1520–1534. [CrossRef] [PubMed]

119. Koh, S.P.; Wickremesekera, A.C.; Brash, H.D.; Marsh, R.; Tan, S.T.; Ittintang, T. Expression of Cathepsins B, D, and G in Isocitrate Dehydrogenase-Wildtype Glioblastoma. *Front. Surg.* 2017, 4, 28. [CrossRef] [PubMed]

120. Basu, S.; Cheryiamundath, S.; Gavert, N.; Brabletz, T.; Haase, G.; Ben-Ze’ev, A. Increased expression of cathepsin D is required for L1-mediated colon cancer progression. *Onco Targets Ther.* 2019, 10, 5217–5228. [CrossRef] [PubMed]

121. Liaudet-Coopman, E.; Beaujouin, M.; Deroçq, D.; Garcia, M.; Glondou-Lassiss, M.; Laurent-Matha, V.; Prébois, C.; Rochefort, H.; Vignon, F. Cathepsin D: Newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. *Cancer Lett.* 2006, 237, 167–179. [CrossRef]

122. Baldwin, E.T.; Bhat, T.N.; Guhnik, S.; Hosur, M.V.; Sovdwer, R.C.; Cachau, R.E.; Collins, J.; Silva, A.M.; Erickson, J.W. Crystal structures of native and inhibited forms of human cathepsin D: Implications for lysosomal targeting and drug design. *Proc. Natl. Acad. Sci. USA* 1993, 90, 6796–6800. [CrossRef] [PubMed]

123. Grädler, U.; Czodrowski, P.; Tsaklakidis, C.; Klein, M.; Werkmann, D.; Lindemann, S.; Maskos, K.; Leuthner, B. Structure-based optimization of non-peptidic Cathepsin D inhibitors. *Bioorg. Med. Chem. Lett.* 2014, 24, 7365–7370. [CrossRef] [PubMed]

124. Ellis, C.R.; Tsai, C.C.; Lin, F.Y.; Shen, J. Conformational dynamics of cathepsin D and binding to a small-molecule BACE1 inhibitor. *Biochem. Biophys. Res. Commun.* 2019, 517, 385–390. [CrossRef] [PubMed]

125. Gan, P.; Ding, L.; Hang, G.; Xia, Q.; Huang, Z.; Ye, X.; Qian, X. Oxymatrine Attenuates Dopaminergic Neuronal Damage in a Parkinson’s Disease Model. *Int. J. Mol. Sci.* 2019, 20, 5170. [CrossRef] [PubMed]

126. Liang, Z.; Liu, Z.; Sun, X.; Tao, M.; Xiao, X.; Yu, G.; Wang, X. The effect of fucoidan on cellular oxidative stress and the CATD-Bax signaling axis in MN9D cells damaged by 1-methyl-4-phenypyridinium. *Front. Aging Neurosci.* 2018, 10, 421. [CrossRef] [PubMed]

127. Singh, M.P.; Sharma, C.; Kang, S.C. Morin hydrate attenuates adenine-induced renal fibrosis via targeting cathepsin D signaling. *Int. Immunopharmacol.* 2021, 90, 107234. [CrossRef] [PubMed]

128. Ellis, C.R.; Tsai, C.C.; Lin, F.Y.; Shen, J. Conformational dynamics of cathepsin D and binding to a small-molecule BACE1 inhibitor. *J. Comput. Chem.* 2017, 38, 1260–1269. [CrossRef]

129. Bagamanshina, A.V.; Soliman, M.E. Hybrid 2D/3D-quantitative structure-activity relationship and modeling studies perspectives of pepstatin A analogs as cathepsin D inhibitors. *Future Med. Chem.* 2018, 10, 5–26. [CrossRef] [PubMed]

130. Jantas, D.; Chwastek, J.; Grygier, B.; Lasoń, W. Neuroprotective Effects of Necrostatin-1 Against Oxidative Stress–Induced Cell Death: An Involvement of Cathepsin D Inhibition. *Front. Pharmacol.* 2020, 11, 776. [CrossRef]

131. Gräf, U.; Czodrowski, P.; Tsaklakidis, C.; Klein, M.; Werkmann, D.; Lindemann, S.; Maskos, K.; Leuthner, B. Structure-based optimization of non-peptidic Cathepsin D inhibitors. *Bioorg. Med. Chem. Lett.* 2017, 28, 675–681. [CrossRef] [PubMed]

132. Jarosz, A.; Kowalczyk, J.; Wasilewski, P.; Wysocki, M.; Nierzbicka, E.; Niedbala, W. Antioxidative and antiinflammatory effects of auranofin-related compounds in human glioblastoma. *J. Neurochem.* 2019, 144, 324–337. [CrossRef] [PubMed]

133. Mikulecky, R.; Faron, K.; Piskorski, M.; Ulrych, M.; Konstantin, D.; Januszkiewicz, A.; Fusaro, V.; Best, R.; Sobolewski, K.; Barcikowski, S. Identification of RNA aptamer which specifically interacts with PtdIns(3)P. *Biochem. Biophys. Res. Commun.* 2020, 524, 155–159. [CrossRef] [PubMed]

134. Jantas, D.; Chwastek, J.; Grygier, B.; Lasoń, W.; Kujawa, K.; Sobolewski, K.; Barcikowski, S. Identification and structure-activity relationship studies of small molecule inhibitors of the human cathepsin D. *Bioorg. Med. Chem. Lett.* 2021, 31, 115879. [CrossRef] [PubMed]

135. Liu, C.M.; Shue, H.T.; Lin, Y.A.; Yu, Y.L.; Chen, Y.; Liu, C.J.; Hsieh, Y.H. Anti-inflamatory and antitumor effects of galectin-3 in human non-small lung cancer through inactivating ERK/CTSD signalling pathways. *Molecules* 2020, 25, 1625. [CrossRef] [PubMed]

136. Bagamanshina, A.V.; Troitskaya, O.S.; Nushtaeva, A.A.; Yunusova, A.Y.; Starykovych, M.O.; Kulagina, E.V.; Kit, Y.Y.; Richter, M.; Wohlfrohm, F.; Kähne, T.; et al. Cytotoxic and antitumor activity of lacticaptin in combination with autophagy inducers and inhibitors. *Biomed Res. Int.* 2019, 2019. [CrossRef] [PubMed]

137. Gan, P.; Xia, Q.; Hang, G.; Zhou, Y.; Qian, X.; Wang, X.; Ding, L. Knockdown of cathepsin D protects dopaminergic neurons against neuroinflammation-mediated neurotoxicity through inhibition of NF-κB signalling pathway in Parkinson’s disease model. *Clin. Exp. Pharmacol. Physiol.* 2019, 46, 337–349. [CrossRef]

138. Donia, T.; Jyoti, B.; Suzui, F.; Hirata, N.; Tanaka, T.; Ishigaki, S.; Pranzatelli, T.J.; Fino-Kobayashi, J.; Iwanga, T.; Chiorini, J.A.; et al. Identification of RNA aptamer which specifically interacts with PtdIns(3)P. *Biochem. Biophys. Res. Commun.* 2019, 517, 146–154. [CrossRef] [PubMed]

139. Cox, J. Cystatins as regulators of cancer. *Med. Res. Arch.* 2017, 5. [CrossRef]

140. Završnik, J.; Butinar, M.; Prebenda, M.T.; Krnjac, A.; Vidmar, R.; Fonović, M.; Grubb, A.; Turk, V.; Turk, B.; Vasiljeva, O. Cystatin C deficiency suppresses tumor growth in a breast cancer model through decreased proliferation of tumor cells. *Oncotarget* 2017, 8, 73793–73809. [CrossRef]

141. Oh, B.M.; Lee, S.-J.; Cho, H.J.; Park, Y.S.; Kim, J.-T.; Yoon, S.R.; Lee, S.C.; Lim, J.-S.; Kim, B.-Y.; Choe, Y.-K.; et al. Cystatin SN inhibits auranofin-induced cell death by autophagic induction and ROS regulation via glutathione reductase activity in colorectal cancer. *Cell Death Dis.* 2017, 8, e2682. [CrossRef] [PubMed]

142. Komura, T.; Takabatake, H.; Harada, K.; Yamato, M.; Miyazawa, M.; Yoshida, K.; Honda, M.; Wada, T.; Kitagawa, H.; Ohta, T.; et al. Clinical features of cystatin A expression in patients with pancreatic ductal adenocarcinoma. *Cancer Sci.* 2017, 108, 2122–2129. [CrossRef] [PubMed]

143. McAdoo, M.H.; Dannenberg, A.M.; Hayes, C.J.; James, S.P.; Sanner, J.H. Inhibition of cathepsin D-type proteinase of macrophages by pepstatin, a specific pepstatin inhibitor, and other substances. *Infect. Immun.* 1973, 7, 655–665. [CrossRef] [PubMed]
142. Musi, M.; Tessitore, L.; Bonelli, G.; Kazakova, O.V.; Baccino, F.M. Changes in rat liver immunoreactive cathepsin D after cycloheximide. *Biochem. Int.* **1985**, *10*, 283–290.

143. Hannapel, D.J. Nucleotide and deduced amino acid sequence of the 22-kilodalton cathepsin D inhibitor protein of potato (*Solanum tuberosum* L.). *Plant Physiol.* **1993**, *101*, 703–704. [CrossRef]

144. Galesa, K.; Pain, R.; Jongsma, M.A.; Turk, V.; Lenarcic, B. Structural characterization of thyroglobulin type-1 domains of equistatin. *FEBS Lett.* **2003**, *539*, 120–124. [CrossRef]

145. Rakitzis, E.T.; Malliopoulou, T.B. Inactivation of cathepsin D by dithiophosgene and by 2,2-dichloro-1,3-dithiacyclobutanone. *Biochem. J.* **1976**, *153*, 737–739. [CrossRef] [PubMed]

146. Kregar, I.; Stanovnik, B.; Tisler, M.; Nisi, C.; Gubensek, F.; Turk, V. Inactivation studies of cathepsin D with diazo compounds. *Acta Biol. Med. Ger.* **1977**, *36*, 1927–1930.

147. Lin, T.Y.; Williams, H.R. Inhibition of cathepsin D by synthetic oligopeptides. *J. Biol. Chem.* **1979**, *254*, 11875–11883. [CrossRef]

148. Gunn, J.M.; Owens, R.A.; Liu, W.S.; Glover, G.I. Biological activity of aspartic proteinase inhibitors related to pepstatin. *Acta Biol. Med. Ger.* **1981**, *40*, 1547–1553. [PubMed]

149. Jupp, R.A.; Dunn, B.M.; Jacobs, J.W.; Vlasuk, G.; Arcuri, K.E.; Veber, D.F.; Perlow, D.S.; Payne, L.S.; Boger, J.; de Laszlo, S. The selectivity of statine-based inhibitors against various human aspartic proteinases. *Biochem. J.* **1990**, *265*, 871–878. [CrossRef]

150. Rao, C.M.; Scarborough, P.E.; Kay, J.; Batley, B.; Rapundalo, S.; Klutchko, S.; Taylor, M.D.; Lunney, E.A.; Humblet, C.C.; Dunn, B.M. Specificity in the binding of inhibitors to the active site of human/primate aspartic proteinases: Analysis of P2-P1-P1’-P2’ variation. *J. Med. Chem.* **1993**, *36*, 2614–2620. [CrossRef]

151. Schulze, H.; Kolter, T.; Sandhoff, K. Principles of lysosomal membrane degradation. Cellular topology and biochemistry of lysosomal lipid degradation. *Biochim. Biophys. Acta Mol. Cell Res.* **2009**, *1793*, 674–683. [CrossRef]

152. Rudzińska, M.; Daglioglu, C.; Savvateneva, L.V.; Kaci, F.N.; Antoine, R.; Zamyatin, A.A. Current Status and Perspectives of Protease Inhibitors and Their Combination with Nanosized Drug Delivery Systems for Targeted Cancer Therapy. *Drug Des. Devel. Ther.* **2021**, *15*, 9–20. [CrossRef] [PubMed]