CHAPTER

10

Cathepsins as an important pharmacological target for multiple cancer types

Thirunavukarasou Anand[1], *Renganathan Senthil*[2], *and Konda Mani Saravanan*[3]

[1]Faculty of Clinical Research, Sri Ramachandra Institute for Higher Education & Research, Chennai, Tamil Nadu, India [2]Department of Bioinformatics, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India [3]Department of Biotechnology, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

10.1 Introduction

Cathepsins (CTS), lysosomal proteases, also known as papain-like enzymes, are members of the C1 family of cysteine proteases (Zaidi et al., 2008). Although CTS show exopeptidase activity, CTS B only functions as an exopeptidase, making cysteine CTS principally endopeptidases (Erickson, 1989). It is understood that such enzymes have functions other than only lysosomal protein breakdown. Evidence shows that CTS reside in other cell structures, such as the nucleus. Furthermore, several clinical diseases verified their existence in the extracellular environment (Yadati et al., 2020). The CTS K, W, and S are more specific, whereas CTS are expressed in human cells (Joyce et al., 2004). The bulk of the epithelial cells, osteoclasts, and synovial fibroblasts in rheumatoid arthritis joints were shown to have CTS K. The immune system's cells express other family members such as CTS S and W (Tan et al., 2013). In particular, CTS S and CTS W were discovered in different kinds of antigen-presenting cells. Finally, only the thymus and testes express CTS V, often known as L2 due to their great homology (Reiser et al., 2010).

Three-dimensional structural analysis of actinidin and papain revealed the highly comparable folds of human CTS B and L (Topham et al., 1991). The determination of the CTS C, H, K, S, V, and X structural details came next (Pauly et al., 2003). According to the claim, CTS molecules are expected to have comparable folds since they share a conservative amino acid sequence (Simões & Faro, 2004). The left (L) and the right domain (R) are the two

Therapeutics of Natural and Synthetic Compounds in Protease-Induced Cancer
DOI: https://doi.org/10.1016/B978-0-443-26635-5.00046-8

domains that make up CTS. CTS L is the representative for this group of proteases. The enzyme's active site cleft, which contains catalytic Cys and His residues, divides its two domains (Stoka et al., 2005). The catalytic Cys residue is located in the L domain, mostly comprised of α-helical regions (Turk et al., 2001). The R domain's structure is described as a β-sheet (Altschuh et al., 1994).

Before becoming fully functional molecules, CTS must undergo further maturation processes after being produced as pre-proenzymes (Brix et al., 2008). This safety measure inhibits the unintentional hydrolysis of targets. The propeptide portion of the enzyme's surface is folded to provide this safety. The propeptide reaches the active-site cleft opposing the substrate, making substrate recruitment difficult (Lazure, 2002). Rough endoplasmic reticulum (ER) is where the majority of CTS are produced (Tedelind et al., 2010). The enzyme molecules are transported across the ER in the subsequent step after entering via the ER lumen. After being processed by the trans-Golgi network, they get organized into vesicles and sent on their way to the late endosomes (Ni et al., 2022). Mannose-6-phosphate receptors, which facilitate the process, separate from the CTS once it reaches its target because of the acidic endosomal location (Laurent-Matha et al., 1998). As in the case of keratinocytes, where CTS are carried through a retrograde process to the cell surface and released into the extracellular space, several different routes may be used to transport CTS to various cell organells (Morretta et al., 2022).

CTS maturation necessitates the removal of the propeptide region. Divergent procedures may result in the elimination. CTS D, an aspartic protease, is one candidate for the proteolytic enzyme that aids in fragment removal (Rochefort et al., 1990). Alternatively, the propeptide might undergo autocatalytic cleavage, creating a functional molecule and so initiating a proteolytic cascade (Maubach et al., 1997). This active molecule might mediate the maturation of another one. This hypothetical idea was tested on CTS B, and it was discovered that the proenzyme can cleave the propeptide region in an acidic environment. It was discovered that negatively charged compounds like glycosaminoglycans or dextran sulfate significantly accelerated the process (Rozman et al., 1999).

Divergent cysteine proteases in this area exhibit considerable differences, as shown by the examination of the fragments' propeptide sequences (Boon et al., 2020). Possible selective inhibition during endosomal compartment trafficking is proposed as a possible explanation for this phenomena (Duffy, 1996). The discovery of a highly conserved ERFNIN motif resulted from an extensive database search of propeptide sequences (Yamamoto et al., 2002). As a result, two sets of cysteine proteases might be formed. The CTS L-like enzymes (L, F, K, S, W, and V) include the ERFNIN motif and the GNFD motif. However, CTS B, C H, O, and X, also known as the CTS B-like subfamily, lack this early motif (Koblinski et al., 2000). It must be made clear that this divide only arises due to variations in the propeptide region.

A groundbreaking investigation carried out by Schechter and Berger in 1967 aimed to identify the catalytic mechanism of CTS (Abramowitz et al., 1967). Papain, regarded as a paradigm instance, was the subject of their study. Schechter and Berger could identify seven pockets in the papain molecule (Turk et al., 1998). Additionally, they established the substrate-enzyme interactions in papain and gave the nomenclature for those locations. P1 to P4 and P1' to P3' refer to the residues of the substrate molecule found in the active site during proteolysis. While primed residues stretch toward the

C-terminus, unprimed residues extend from the cleavage point to the amino-terminal side (Templeton et al., 1990). The L-domain's two protruding loops, linked by a disulfide bridge, and the R-domain's two bigger loops make up the substrate-binding site. The substrate's prolonged conformational binding was shown. The S1 and S1' subsites, along with S2 provide the binding surface and deep pocket. In general, CTS have a low degree of specificity because the S3 and S4 subsites are extremely nonspecific, even though they favor cleavage after hydrophobic residues (Benes et al., 2008). The discovery of CTS-specific substrates would make the development of more effective inhibitors possible. Although recent research has favored noncovalent transition state mimics such as phosphopeptides, most known CTS inhibitors work by covalently attaching to the catalytic cysteine residue (Löser & Pietzsch, 2015).

10.2 Role of cathepsins in apoptosis

According to recent studies, CTS may be crucial in the control of apoptosis (Chwieralski et al., 2006). It has been shown that they can interact with caspases leading to programmed cell death. CTS must be released from lysosomes into the cytosol to perform proapoptotic actions (Leist & Jäättelä, 2001). Lysosomal instability or lysosomal membrane permeabilization may both be used to accomplish this. Despite the discovery of a number of chemicals that target the lysosomal membrane, the latter process is the subject of intensive study at the present time (Saravanan et al., 2022; Vasiljeva & Turk, 2008). These include the well-known tumor necrosis factor and antitumorigenic substances. Lysosomotropic substances include sphingosine, L-leucyl-L-leucine methyl ester, and O-methyl-serine dodecylamide hydrochloride in a separate category (Conus & Simon, 2008).

CTS were shown to have intracellular apoptosis-related targets (Mustafa et al., 2023). Bid, a member of the Bcl-2 family and a key player in the intrinsic route of apoptosis' permeabilization of the mitochondrial membrane was the first to be characterized (Vila-Julià et al., 2023). The finding that inhibiting the release of cytochrome c after incubation of mitochondria from Bid-deficient cell lines supported the idea that processing of Bid could be important in lysosomal CTS-mediated apoptosis. Uncertainty persists about the function of lysosomal membrane permeabilization in programmed cell death (Patra et al., 2023). The main point of contention is whether permeabilization happens before or after the proteolytic, apoptotic cascade. Caspase-8 might contribute to the rupture of the lysosomal membrane in response to death stimuli, which might support the upstream notion (Zhong et al., 2020).

10.3 Role of cathepsins in different cancer types

Although CTS participation in apoptosis may be a positive indication that their deficiency might result in evasion, their role in altering the extracellular matrix is likely considerably significant (Zuzarte-Luis et al., 2007). This mechanism controls the organism's growth and

homeostasis preservation. But if changed, it may have detrimental pathological effects (Baici et al., 2006; Le et al., 2021). Environmental and cellular signals have a role in cancer's progression and dynamic character. In numerous common human malignancies, increased CTS levels and reduced CTS inhibitor levels have been documented (Konduri et al., 2001). Metastases, which are today a significant cause of mortality in cancer patients, arise as a result of the process ineluctably (Vizovišek et al., 2019). The CTS translocation mechanisms are disrupted during carcinogenesis, which causes the enzyme molecules to move onto specialized areas of the cell surface. Because of this, malignant cells' proteases may cleave otherwise inaccessible targets (Birkedal-Hansen, 1993). Numerous intricate interactions between diverse variables result in the proteolytic network that drives tumor development. CTS also destroy osteocin and other matricellular proteins that are not structural components of the extracellular matrix (Góra & Latajka, 2015).

10.3.1 Cathepsins B and L in cancer

The CTS B and L are most commonly referenced in papers about the study of cancer among the whole CTS family. Multiple cancer forms have abnormal expressions and patterns of CTS (Li et al., 2017; Sundaram et al., 2022). According to research by Werle et al., CTS B and L activity is elevated in lung cancer tissue (Xing et al., 1998). A relatively sizable sample of lung cancer patients participated in the case-control research. CTS L activity was measured using the specific substrate Z-Phe-Arg-AMC. To further guarantee accurate results and prevent their deterioration, CA-074 was launched as a CTS B inhibitor. Similarly, the CTS B activity was evaluated in the sera of lung cancer patients assessed in another investigation (Chen et al., 2011). It has been shown that patients who have high levels of both CTS B and cystatin C have a much worse survival rate than other test participants. Patients with prostate cancer also had abnormalities in CTS B and L (Jedeszko & Sloane, 2004). Fernández et al. (2001) concluded that CTS B and S may be crucial in the invasion of prostate cancers. Additional reports of aberrant CTS B have been made in the context of a different prevalent human malignancy, colorectal cancer. Intraepithelial neoplasms are the primary cause of most invasive colorectal malignancies. In an indirect attempt to determine CTS B levels in individuals with colorectal cancer, the levels of CTS B-specific antigen were examined in 64 cases and compared with immunohistochemistry labeling of controls from background colon tissue (Hirai et al., 1999). It has been shown that cancer tissue has considerably more CTS B antigen than the comparable normal mucosa. In a set of paired malignant and adjacent normal colorectal tissues, the cytosolic concentration of CTS B and L was compared by Adenis et al. (1995). Activated cathepsin L in complex with its propeptide is shown in Fig. 10.1. They demonstrated that the CTS levels are higher than those in the nearby, nonmalignant tissues. However, they could not link the variations in expression to gender, age, location, or stage of the disease. However, Hirai and coworkers have shown that CTS B expression is increased in colorectal carcinomas by 3.7-fold at the mRNA level (Hirai et al., 1999). Throughout their investigation, they examined the distribution of CTS B in 80 patients' malignant and normal tissues. CTS B expression was correlated with colon cancer survival by Chan et al. (2010). 457 of the 558 people who took part in their study had malignancies that were CTSB positive. Furthermore, it was shown that the

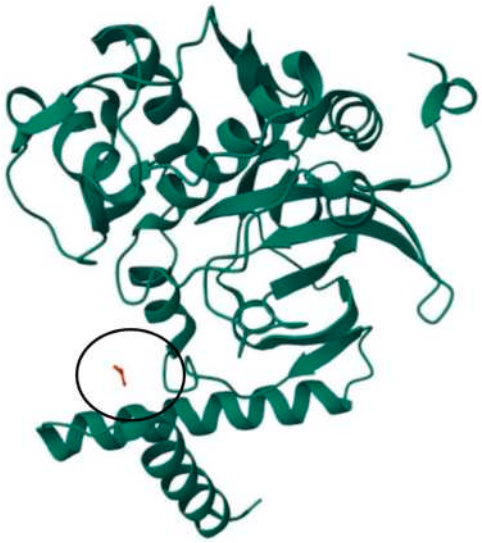

FIGURE 10.1 Activated cathepsin (CTS) L in complex with its propeptide. The Protein Databank identifier is 3F75. The CTS L is shown as a cartoon whereas propeptide is shown as sticks. Visualization of activated CTS L in complex with its propeptide, as represented by Protein Data Bank identifier 3F75. The figure depicts CTS L as a cartoon model, highlighting its overall structure and active site, while the propeptide is shown as stick models, illustrating its precise interaction with the enzyme. This complex formation is crucial for regulating CTS L activity and its role in various physiological and pathological processes.

expression pattern was unrelated to the tumor stage. They found that overall mortality and a greater risk of colon cancer are associated with CTSB expression. Additionally, CTS B has been identified as a key player in encouraging cell proliferation and the development of distant metastases, making it an enzyme of paramount significance in different forms of cancer. According to research, CTS B silencing prevents growth (Bao et al., 2013) (Fig. 10.1).

Compared to normal endometrium and endometrial atypical hyperplasia tissues, cancerous endometrial tissues were shown to have significantly elevated levels of CTS B. Based on these results, CTS B was identified as a potential therapeutic target for the treatment of endometrial cancer. Three single-nucleotide polymorphisms (SNPs) in the CTS B encoding genes were also evaluated for their role in carcinogen-induced mouth cancer susceptibility (Chen et al., 2012). All three abnormalities were shown to have a substantial impact on the probability of developing mouth cancer. There was a strong correlation between one SNP and an increased likelihood of oral cancer developing clinicopathologically, and another SNP may dramatically increase sensitivity to environmental carcinogen-mediated oral cancer. In the context of breast cancer, the function of CTS B inhibition was also investigated (Withana et al., 2012). According to the findings, inhibiting CTS B activity with CA-074 prevents bone metastases in vivo and reduces collagen I degradation in vitro. The degree of CTS B expression was also linked with the depth of tumor invasion and lymphatic metastasis in cervical cancer (Wu et al., 2012). The investigation used 169 clinical

samples in a series. According to the findings, CTS B production is noticeably greater and may be used as a diagnostic for cervical cancer.

10.3.2 Role of cathepsins K

In normal situations, the CTS K enzyme helps with bone rebuilding and resorption, and it has a high amount of kinin affinity that sets it apart. However, studies of CTS K's aberrant expression and its connection to many malignancies may be found in the literature (Kawai et al., 2023). A team led by Bühling looked into how CTS K is expressed in the lungs and airways (Bühling et al., 2002). Using quantitative RT-PCR, they established that the levels of CTS K-mRNA are increased in lung cancer tissues. Since CTS K actively contributes to bone remodeling and degeneration and prostate cancer often exhibits enhanced bone formation and resorption, its probable involvement in prostate cancer was widely predicted. Cathepsin K complexed with a cyanamide-based inhibitor is shown in Fig. 10.2. Another research have reported anomalies associated with CTS K in cases of prostate cancer (Jedeszko & Sloane, 2004). Additionally, a team headed by Brubaker looked at the role of CTS K in prostate

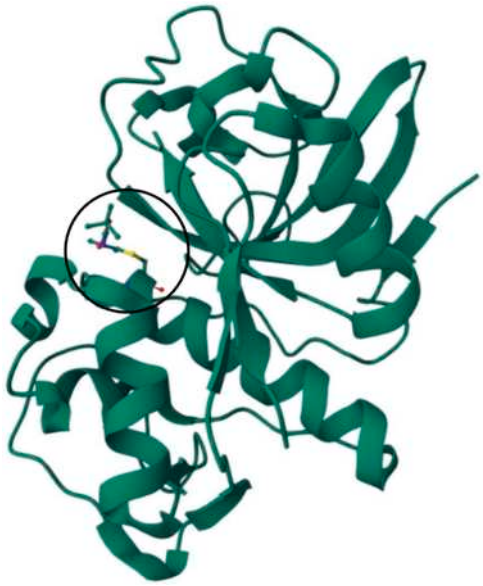

FIGURE 10.2 Cathepsin (CTS) K complexed with a cyanamide-based inhibitor. The Protein Databank identifier is 1YK8. The CTS K is shown as cartoon model whereas ligand is shown as sticks. Depiction of CTS K complexed with a cyanamide-based inhibitor, based on Protein Data Bank identifier 1YK8. The figure shows CTS K as a cartoon model, providing a detailed view of its three-dimensional structure and active site, while the cyanamide-based inhibitor is represented as a stick model, illustrating its binding interactions within the enzyme's active site. This complex showcases the inhibitor's mode of action and its potential for therapeutic targeting of CTS K in various diseases.

cancer (Bühling et al., no date). RT-PCR was utilized in their work to analyze the mRNA fragments quantitatively. The accumulated data showed higher CTS K levels in cancer tissue, consistent with the original premise.

10.4 Other important types of cathepsins

Members of the CTS family that are less often expressed, such as CTS H, S, and X, have also been linked to cancer instances. Using ELISA as the preferred technique, Schweiger et al. explored the function of CTS H in lung cancer (Schweiger et al., 2000). The amounts of CTS H were tested in 171 people with cancerous tumors, 34 people with harmless lung diseases, and 47 healthy people who were used as standards. The results show that CTS H is expressed 0.64 times more in cancer tissue than in normal lung tissue. Also, they have shown that people with high amounts of CTS H in their tumors who smoke have a lower chance of living longer than people who don't smoke. Waghray and colleagues (Waghray et al., 2002) also observed a considerable rise in CTS H expression in neoplasia carcinoma. They also used cDNA probes to find a shortened variant of CTS H in the tumor environment. They uncovered evidence throughout the research that deletion will likely happen at the RNA processing level. Eventually, the loss inside the propeptide segment was identified as the cause of the CTS H trafficking problems. In 2000, an analysis of CTS H protein types and activity levels in human colorectal cancer was conducted (Re et al., 2000). Seventy-seven people with colorectal cancer participated in the research. It was determined that tumor cells have enhanced CTS H-specific activity. A relationship between the tumor stage and CTS H level was also established. For example, CTS H levels were commonly reported to be high in the C-stage carcinomas from Duke. Kos et al. (2001) examined the amounts of CTS S in the tissue cytosols of lymph nodes and lung tumors. The tumor cells were from 11 instances of secondary tumors and 62 individuals with nonsmall cell lung cancer. The study of the samples revealed that tumor tissue had a 1.5-fold higher expression of CTS S than the nearby, noncancerous tissues. According to other investigations, CTS S may be involved in instances of prostate cancer (Fernández et al., 2001). Finally, it is important to note that 56 men who had radical therapy for prostate cancer had matched malignant and nonmalignant tissues evaluated to assess the significance of CTS X in prostate cancers (Nägler et al., 2004). Multiple techniques were used in the study to quantify enzyme expression: RT-PCR, in situ hybridization, immunohistochemistry, and Western blotting. The findings demonstrate that the CTS X-chromosome region is often amplified in prostate cancer, consistent with increased CTS expression.

10.5 Conclusion

Members of the protease family are crucial to several crucial biological activities. Numerous studies have shown that different family members were involved in various cancer instances. In reality, it is evident when one considers CTS involvement. Numerous

data suggest that various malignancies have increased CTS levels and activity. The CTS B and L are the two members that get the greatest attention. However, other members of this family of enzymes may also be discovered to be the focus of cancer research. Most of the most common types of human cancer, including colorectal, prostate, and lung malignancies, showed abnormal CTS expression levels. Aberrant caspases aid in evading apoptosis, but elevated CTS levels play a different role in tumor formation. Consequently, CTS are crucial in the migration of malignant cells.

Given that CTS have established themselves as important biomarkers for cancer, we may predict that they will soon be widely employed in diagnostics and early disease detection. Additionally, CTS B-cleavable chemotherapeutic drugs are an important area of future medical study. Due to the limited substrate specificity of this class of enzymes, it is difficult to construct highly selective CTS inhibitors. However, computational investigation of the binding pockets of several CTS family members may help with this. Identifying new lead compounds may be accomplished theoretically using quantum chemistry or molecular dynamics techniques, often less expensive than experimental procedures. This indicates that they have a role in developing metastases, constituting a significant obstacle in the battle against cancer. Based on the information acquired, we believe that CTS should be prioritized in tumor-related research since they may serve as therapeutic targets or prognostic indicators in cancer treatment. Cysteine proteases are used in prognostics in a very simple manner. Overall, it is obvious that cysteine proteases play a role in cancer, and this fact must be considered in any future approaches to cancer therapy, diagnosis, and prognosis.

References

Abramowitz, N., Schechter, I., & Berger, A. (1967). On the size of the active site in proteases II. Carboxypeptidase-A. *Biochemical and Biophysical Research Communications, 29*(6), 862–867. https://doi.org/10.1016/0006-291x(67)90299-9.

Adenis, A., Huet, G., Zerimech, F., Hecquet, B., Balduyck, M., & Peyrat, J. P. (1995). Cathepsin B, L, and D activities in colorectal carcinomas: Relationship with clinico-pathological parameters. *Cancer Letters, 96*(2), 267–275. https://doi.org/10.1016/0304-3835(95)03930-u.

Altschuh, D., Tessier, D. C., & Vernet, T. (1994). Modulation of the enzymatic activity of papain by interdomain residues remote from the active site. *Protein Engineering, Design and Selection, 7*(6), 769–776. https://doi.org/10.1093/protein/7.6.769.

Baici, A., Müntener, K., Willimann, A., & Zwicky, R. (2006). Regulation of human cathepsin B by alternative mRNA splicing: Homeostasis, fatal errors and cell death. *Biological Chemistry, 387*(8), 1017–1021. https://doi.org/10.1515/BC.2006.125.

Bao, W., Fan, Q., Luo, X., Cheng, W. W., Wang, Y. D., Li, Z. N., Chen, X. L., & Wu, D. (2013). Silencing of cathepsin B suppresses the proliferation and invasion of endometrial cancer. *Oncology Reports. 30*(2), 723–730. https://doi.org/10.3892/or.2013.2496.

Benes, P., Vetvicka, V., & Fusek, M. (2008). Cathepsin D—Many functions of one aspartic protease. *Critical Reviews in Oncology/Hematology, 68*(1), 12–28. https://doi.org/10.1016/j.critrevonc.2008.02.008.

Birkedal-Hansen, H. (1993). Role of cytokines and inflammatory mediators in tissue destruction. *Journal of Periodontal Research, 28*(7), 500–510. https://doi.org/10.1111/j.1600-0765.1993.tb02113.x.

Boon, L., Ugarte-Berzal, E., Vandooren, J., & Opdenakker, G. (2020). Protease propeptide structures, mechanisms of activation, and functions. *Critical Reviews in Biochemistry and Molecular Biology, 55*(2), 111–165. https://doi.org/10.1080/10409238.2020.1742090.

Brix, K., Dunkhorst, A., Mayer, K., & Jordans, S. (2008). Cysteine cathepsins: Cellular roadmap to different functions. *Biochimie, 90*(2), 194–207. https://doi.org/10.1016/j.biochi.2007.07.024.

Bühling, F., Waldburg, N., Gerber, A., Häckel, C., Krüger, S., Reinhold, D., Brömme, D., Weber, E., Ansorge, S., & Welte, T. (2002). *Cathepsin K expression in human lung*. Springer Science and Business Media LLC. https://doi.org/10.1007/0-306-46826-3_30.

Chan, A. T., Baba, Y., Shima, K., Nosho, K., Chung, D. C., Hung, K. E., Mahmood, U., Madden, K., Poss, K., Ranieri, A., Shue, D., Kucherlapati, R., Fuchs, C. S., & Ogino, S. (2010). Cathepsin B expression and survival in colon cancer: Implications for molecular detection of neoplasia. *Cancer Epidemiology Biomarkers and Prevention, 19*(11), 2777–2785. https://doi.org/10.1158/1055-9965.EPI-10-0529.

Chen, M. K., Su, S. C., Lin, C. W., Tsai, C. M., Yang, S. F., & Weng, C. J. (2012). Cathepsin B SNPs elevate the pathological development of oral cancer and raise the susceptibility to carcinogen-mediated oral cancer. *Human Genetics, 131*(12), 1861–1868. https://doi.org/10.1007/s00439-012-1211-1.

Chen, Q., Fei, J., Wu, L., Jiang, Z., Wu, Y., Zheng, Y., & Lu, G. (2011). Detection of cathepsin B, cathepsin L, cystatin C, urokinase plasminogen activator and urokinase plasminogen activator receptor in the sera of lung cancer patients. *Oncology Letters, 2*(4), 693–699. https://doi.org/10.3892/ol.2011.302.

Chwieralski, C. E., Welte, T., & Bühling, F. (2006). Cathepsin-regulated apoptosis. *Apoptosis: an International Journal on Programmed Cell Death, 11*(2), 143–149. https://doi.org/10.1007/s10495-006-3486-y.

Conus, S., & Simon, H. U. (2008). Cathepsins: Key modulators of cell death and inflammatory responses. *Biochemical Pharmacology, 76*(11), 1374–1382. https://doi.org/10.1016/j.bcp.2008.07.041.

Duffy, M. J. (1996). Proteases as prognostic markers in cancer. *Clinical Cancer Research, 2*(4), 613–618.

Erickson, A. H. (1989). Biosynthesis of lysosomal endopeptidases. *Journal of Cellular Biochemistry, 40*(1), 31–41. https://doi.org/10.1002/jcb.240400104.

Fernández, P. L., Farré, X., Nadal, A., Fernández, E., Peiró, N., Sloane, B. F., Shi, G. P., Chapman, H. A., Campo, Ea, & Cardesa, A. (2001). Expression of Cathepsins B and S in the progression of prostate carcinoma. *International Journal of Cancer, 95*(1), 51–55. https://doi.org/10.1002/1097-0215(20010120)95:1<51::AID-IJC1009>3.0.CO;2-J.

Góra, J., & Latajka, R. (2015). Involvement of cysteine proteases in cancer. *Current Medicinal Chemistry, 22*(8), 944–957. https://doi.org/10.2174/0929867321666141106115624.

Hirai, K., Yokoyama, M., Asano, G., & Tanaka, S. (1999). Expression of cathepsin B and cystatin C in human colorectal cancer. *Human Pathology, 30*(6), 680–686. https://doi.org/10.1016/s0046-8177(99)90094-1.

Jedeszko, C., & Sloane, B. F. (2004). Cysteine cathepsins in human cancer. *Biological Chemistry, 385*(11), 1017–1027. https://doi.org/10.1515/BC.2004.132.

Joyce, J. A., Baruch, A., Chehade, K., Meyer-Morse, N., Giraudo, E., Tsai, F. Y., Greenbaum, D. C., Hager, J. H., Bogyo, M., & Hanahan, D. (2004). Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. *Cancer Cell, 5*(5), 443–453. https://doi.org/10.1016/S1535-6108(04)00111-4.

Kawai, R., Sugisaki, R., Miyamoto, Y., Yano, F., Sasa, K., Minami, E., Maki, K., & Kamijo, R. (2023). Cathepsin K degrades osteoprotegerin to promote osteoclastogenesis in vitro. *In Vitro Cellular & Developmental Biology - Animal, 59*(1), 10–18. https://doi.org/10.1007/s11626-023-00747-5.

Koblinski, J. E., Ahram, M., & Sloane, B. F. (2000). Unraveling the role of proteases in cancer. *Clinica Chimica Acta, 291*(2), 113–135. https://doi.org/10.1016/S0009-8981(99)00224-7.

Konduri, S., Lakka, S. S., Tasiou, A., Yanamandra, N., Gondi, C. S., Dinh, D. H., Olivero, W. C., Gujrati, M., & Rao, J. S. (2001). Elevated levels of cathepsin B in human glioblastoma cell lines. *International Journal of Oncology, 19*(3), 519–524. https://doi.org/10.3892/ijo.19.3.519.

Kos, J., Sekirnik, A., Kopitar, G., Cimerman, N., Kayser, K., Stremmer, A., Fiehn, W., & Werle, B. (2001). Cathepsin S in tumours, regional lymph nodes and sera of patients with lung cancer: Relation to prognosis. *British Journal of Cancer, 85*(8), 1193–1200. https://doi.org/10.1054/bjoc.2001.2057.

Laurent-Matha, V., Farnoud, M. R., Lucas, A., Rougeot, C., Garcia, M., & Rochefort, H. (1998). Endocytosis of pro-cathepsin D into breast cancer cells is mostly independent of mannose-6-phosphate receptors. *Journal of Cell Science, 111*(17), 2539–2549. https://doi.org/10.1242/jcs.111.17.2539.

Lazure, C. (2002). The peptidase zymogen proregions: Natures way of preventing undesired activation and proteolysis. *Current Pharmaceutical Design, 8*(7), 511–531. https://doi.org/10.2174/1381612023395691.

Le, H. T. T., Murugesan, A., Ramesh, T., Yli-Harja, O., Konda Mani, S., & Kandhavelu, M. (2021). Molecular interaction of HIC, an agonist of P2Y1 receptor, and its role in prostate cancer apoptosis. *International Journal of Biological Macromolecules, 189*, 142–150. https://doi.org/10.1016/j.ijbiomac.2021.08.103.

Leist, M., & Jäättelä, M. (2001). Triggering of apoptosis by cathepsins. *Cell Death & Differentiation, 8*(4), 324–326. https://doi.org/10.1038/sj.cdd.4400859.

Li, Y. Y., Fang, J., & Ao, G. Z. (2017). Cathepsin B and L inhibitors: A patent review (2010 - present. *Expert Opinion on Therapeutic Patents, 27*(6), 643–656. https://doi.org/10.1080/13543776.2017.1272572.

Löser, R., & Pietzsch, J. (2015). Cysteine cathepsins: Their role in tumor progression and recent trends in the development of imaging probes. *Frontiers in Chemistry, 3*, 37. https://doi.org/10.3389/fchem.2015.00037.

Maubach, G., Schilling, K., Rommerskirch, W., Wenz, I., Schultz, J. E., Weber, E., & Wiederanders, B. (1997). The inhibition of cathepsin S by its propeptide—Specificity and mechanism of action. *European Journal of Biochemistry, 250*(3), 745–750. https://doi.org/10.1111/j.1432-1033.1997.00745.x.

Morretta, E., D'agostino, A., Cassese, E., Maglione, B., Petrella, A., Schiraldi, C., & Monti, M. C. (2022). Label-free quantitative proteomics to explore the action mechanism of the pharmaceutical-grade *Triticum vulgare* extract in speeding up keratinocyte healing. *Molecules (Basel, Switzerland), 27*(3), 1108. https://doi.org/10.3390/molecules27031108.

Mustafa, A., Elkhamisy, F., Arghiani, N., & Pranjol, M. Z. I. (2023). Potential crosstalk between pericytes and cathepsins in the tumour microenvironment. *Biomedicine & Pharmacotherapy, 164*, 114932. https://doi.org/10.1016/j.biopha.2023.114932.

Nägler, D. K., Krüger, S., Kellner, A., Ziomek, E., Menard, R., Buhtz, P., Krams, M., Roessner, A., & Kellner, U. (2004). Up-regulation of cathepsin X in prostate cancer and prostatic intraepithelial neoplasia. *The Prostate, 60*(2), 109–119. https://doi.org/10.1002/pros.20046.

Ni, J., Lan, F., Xu, Y., Nakanishi, H., & Li, X. (2022). Extralysosomal cathepsin B in central nervous system: Mechanisms and therapeutic implications. *Brain Pathology, 32*(5), e13071. https://doi.org/10.1111/bpa.13071.

Patra, S., Patil, S., Klionsky, D. J., & Bhutia, S. K. (2023). Lysosome signaling in cell survival and programmed cell death for cellular homeostasis. *Journal of Cellular Physiology, 238*(2), 287–305. https://doi.org/10.1002/jcp.30928.

Pauly, T. A., Sulea, T., Ammirati, M., Sivaraman, J., Danley, D. E., Griffor, M. C., Kamath, A. V., Wang, I. K., Laird, E. R., Seddon, A. P., Ménard, R., Cygler, M., & Rath, V. L. (2003). Specificity determinants of human cathepsin S revealed by crystal structures of complexes. *Biochemistry, 42*(11), 3203–3213. https://doi.org/10.1021/bi027308i.

Re, E. C.d, Shuja, S., Cai, J., & Murnane, M. J. (2000). Alterations in cathepsin H activity and protein patterns in human colorectal carcinomas. *British Journal of Cancer, 82*(7), 1317–1326. https://doi.org/10.1054/bjoc.1999.1098.

Reiser, J., Adair, B., & Reinheckel, T. (2010). Specialized roles for cysteine cathepsins in health and disease. *Journal of Clinical Investigation, 120*(10), 3421–3431. https://doi.org/10.1172/jci42918.

Rochefort, H., Capony, F., & Garcia, M. (1990). Cathepsin D: A protease involved in breast cancer metastasis. *Cancer and Metastasis Review, 9*(4), 321–331. https://doi.org/10.1007/bf00049522.

Rozman, J., Stojan, J., Kuhelj, R., Turk, V., & Turk, B. (1999). Autocatalytic processing of recombinant human procathepsin B is a bimolecular process. *FEBS Letters, 459*(3), 358–362. https://doi.org/10.1016/s0014-5793(99)01302-2.

Saravanan, K. M., Kannan, M., Meera, P., Bharathkumar, N., & Anand, T. (2022). E3 ligases: A potential multi-drug target for different types of cancers and neurological disorders. *Future Medicinal Chemistry, 14*(3), 187–201. https://doi.org/10.4155/fmc-2021-0157.

Schweiger, A., Staib, A., Werle, B., Kra[sbreve]ovec, M., Lah, T. T., Ebert, W., Turk, V., & Kos, J. (2000). Cysteine proteinase cathepsin H in tumours and sera of lung cancer patients: Relation to prognosis and cigarette smoking. *British Journal of Cancer, 82*(4), 782–788. https://doi.org/10.1054/bjoc.1999.0999.

Simões, I., & Faro, C. (2004). Structure and function of plant aspartic proteinases. *European Journal of Biochemistry, 271*(11), 2067–2075. https://doi.org/10.1111/j.1432-1033.2004.04136.x.

Stoka, V., Turk, B., & Turk, V. (2005). Lysosomal cysteine proteases: Structural features and their role in apoptosis. *IUBMB Life, 57*(4-5), 347–353. https://doi.org/10.1080/15216540500154920.

Sundaram, K. K. M., Bupesh, G., & Saravanan, K. M. (2022). Instrumentals behind embryo and cancer: A platform for prospective future in cancer research. *AIMS Molecular Science, 9*(1), 25–45. https://doi.org/10.3934/molsci.2022002.

Tan, G.-J., Peng, Z.-K., Lu, J.-P., & Tang, F.-Q. (2013). Cathepsins mediate tumor metastasis. *World Journal of Biological Chemistry, 4*(4), 91. https://doi.org/10.4331/wjbc.v4.i4.91.

Tedelind, S., Poliakova, K., Valeta, A., Hunegnaw, R., Yemanaberhan, E. L., Heldin, N. E., Kurebayashi, J., Weber, E., Kopitar-Jerala, N., Turk, B., Bogyo, M., & Brix, K. (2010). Nuclear cysteine cathepsin variants in thyroid carcinoma cells. *Biological Chemistry, 391*(8), 923–935. https://doi.org/10.1515/BC.2010.109.

Templeton, W., Kowlessur, D., Thomas, E. W., Topham, C. M., & Brocklehurst, K. (1990). A re-appraisal of the structural basis of stereochemical recognition in papain. Insensitivity of binding-site-catalytic-site signalling to P2-chirality in a time-dependent inhibition. *Biochemical Journal, 266*(3), 645–651. https://doi.org/10.1042/bj2660645.

Topham, C. M., Salih, E., Frazao, C., Kowlessur, D., Overington, J. P., Thomas, M., Brocklehurst, S. M., Patel, M., Thomas, E. W., & Brocklehurst, K. (1991). Structure-function relationships in the cysteine proteinases actinidin, papain and papaya proteinase Ω. Three-dimensional structure of papaya proteinase Ω deduced by knowledge-based modelling and active-centre characteristics determined by two-hydronic-state reactivity probe kinetics and kinetics of catalysis. *Biochemical Journal, 280*(1), 79–92. https://doi.org/10.1042/bj2800079.

Turk, D., Gunčar, G., Podobnik, M., & Turk, B. (1998). Revised definition of substrate binding sites of papain-like cysteine proteases. *bchm, 379*(2), 137–148. https://doi.org/10.1515/bchm.1998.379.2.137.

Turk, V., Turk, B., & Turk, D. (2001). Lysosomal cysteine proteases: Facts and opportunities. *EMBO Journal, 20*(17), 4629–4633. https://doi.org/10.1093/emboj/20.17.4629.

Vasiljeva, O., & Turk, B. (2008). Dual contrasting roles of cysteine cathepsins in cancer progression: Apoptosis versus tumour invasion. *Biochimie, 90*(2), 380–386. https://doi.org/10.1016/j.biochi.2007.10.004.

Vila-Julià, G., Perez, J. J., & Rubio-Martinez, J. (2023). A step forward toward selective activation/inhibition of bak, a pro-apoptotic member of the Bcl-2 protein family: discovery of new prospective allosteric sites using molecular dynamics. *Journal of Chemical Information and Modeling, 63*(11), 3544–3556. https://doi.org/10.1021/acs.jcim.3c00397.

Vizovišek, M., Fonović, M., & Turk, B. (2019). Cysteine cathepsins in extracellular matrix remodeling: Extracellular matrix degradation and beyond. *Matrix Biology, 75-76*, 141–159. https://doi.org/10.1016/j.matbio.2018.01.024.

Waghray, A., Keppler, D., Sloane, B. F., Schuger, L., & Chen, Y. Q. (2002). Analysis of a truncated form of cathepsin H in human prostate tumor cells. *Journal of Biological Chemistry, 277*(13), 11533–11538. https://doi.org/10.1074/jbc.M109557200.

Withana, N. P., Blum, G., Sameni, M., Slaney, C., Anbalagan, A., Olive, M. B., Bidwell, B. N., Edgington, L., Wang, L., Moin, K., Sloane, B. F., Anderson, R. L., Bogyo, M. S., & Parker, B. S. (2012). Cathepsin B inhibition limits bone metastasis in breast cancer. *Cancer Research, 72*(5), 1199–1209. https://doi.org/10.1158/0008-5472.CAN-11-2759.

Wu, D., Wang, H., Li, Z., Wang, L., Zheng, F., Jiang, J., Gao, Y., Zhong, H., Huang, Y., & Suo, Z. (2012). Cathepsin B may be a potential biomarker in cervical cancer. *Histology and Histopathology, 27*(1), 79–87.

Xing, R., Addington, A. K., & Mason, R. W. (1998). Quantification of cathepsins B and L in cells. *Biochemical Journal, 332*(2), 499–505. https://doi.org/10.1042/bj3320499.

Yadati, T., Houben, T., Bitorina, A., & Shiri-Sverdlov, R. (2020). The ins and outs of cathepsins: Physiological function and role in disease management. *Cells, 9*(7), 1679. https://doi.org/10.3390/cells9071679.

Yamamoto, Y., Kurata, M., Watabe, S., Murakami, R., & Takahashi, S. (2002). Novel cysteine proteinase inhibitors homologous to the proregions of cysteine proteinases. *Current Protein & Peptide Science, 3*(2), 231–238. https://doi.org/10.2174/1389203024605331.

Zaidi, N., Maurer, A., Nieke, S., & Kalbacher, H. (2008). Cathepsin D: A cellular roadmap. *Biochemical and Biophysical Research Communications, 376*(1), 5–9. https://doi.org/10.1016/j.bbrc.2008.08.099.

Zhong, B., Liu, M., Bai, C., Ruan, Y., Wang, Y., Qiu, L., Hong, Y., Wang, X., Li, L., & Li, B. (2020). Caspase-8 induces lysosome-associated cell death in cancer cells. *Molecular Therapy, 28*(4), 1078–1091. https://doi.org/10.1016/j.ymthe.2020.01.022.

Zuzarte-Luis, V., Montero, J. A., Kawakami, Y., Izpisua-Belmonte, J. C., & Hurle, J. M. (2007). Lysosomal cathepsins in embryonic programmed cell death. *Developmental Biology, 301*(1), 205–217. https://doi.org/10.1016/j.ydbio.2006.08.008.