Chapter: Cathepsin D in the tumor microenvironment of breast and ovarian cancers

Md Zahidul I. Pranjol [1]*, Jacqueline L. Whatmore [2]

1. William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK.
2. Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, Devon EX1 2LU, UK.

* Corresponding author: William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK.

Email address: z.pranjol@qmul.ac.uk

Md Zahidul I. Pranjol z.pranjol@qmul.ac.uk

Jacqueline L. Whatmore J.L.Whatmore@exeter.ac.uk

Abstract

Cancer remains a major and leading health problem worldwide. Lack of early diagnosis, chemoresistance and recurrence of cancer means vast research and development are required in this area. The complexity of the tumor microenvironment in the biological milieu poses greater challenges in having safer, selective and targeted therapies. Existing strategies such as chemotherapy, radiotherapy and anti-angiogenic therapies moderately improve progression-free survival, however, they come with side-effects that reduce quality of life. Thus, targeting potential candidates in the microenvironment, such as extracellular cathepsin D (CathD) which has been known to play major pro-tumorigenic roles in breast and ovarian cancers, could be a breakthrough in cancer treatment, specially using novel treatment modalities such as immunotherapy and nanotechnology-based therapy. This chapter discusses CathD as a pro-cancerous, more specifically a proangiogenic factor, that acts bi-functionally in the tumor microenvironment, and possible ways of targeting the protein therapeutically.

Introduction

Globally, more than 2.28 million new cases of breast and ovarian cancers are diagnosed, with approximately 810,000 deaths each year [1-3]. Thus, tackling these two major cancers remains a daunting task for clinicians and researchers. By the year 2025, it is estimated that, globally, there will be a surge in the number of cancer cases (>20 million annually) - an alarming statistic that has compelled researchers to expedite research to discover newer targets and develop more potent therapeutic compounds to overcome drug resistance as well as eradicate cancer cells from the biological setting [4]. However, the disease remains a global challenge due to the lack of early diagnosis, the inherent biological complexity and the high demands for designing safer and selective drugs to restrict tumor growth [5].

Although researchers have a much better understanding of many characteristics of cancer [6], the complex systems that allow tumors to form remains to be solved. It is the complex crosstalk between the cellular and non-cellular components of the host organ which, under the influence of the tumor cells, help create a niche for tumors to grow uncontrollably, invade local tissue, evade local immune-mediated destruction, and stimulate angiogenesis and metastasis [7]. This newly formed niche where tumors sit and grow is known as the tumor microenvironment **(Figure 1)**. A number of cells, such as cancer-associated fibroblasts (CAFs), immune cells, adipocytes, neuroendocrine cells, the blood, and lymphatic vascular networks, and tumor cells, help build this niche [8]. Once tumors start to grow in this hypoxic microenvironment, where the normal cell and tissue homeostasis is dysregulated, they secrete both pro- and anti-tumorigenic growth factors, cytokines, extracellular vesicles, extracellular matrix (ECM) proteins and ECM-remodeling enzymes that trigger a switch to a more pro-tumorigenic response from the surrounding cells [7]. For instance, CAFs, pericytes, endothelial cells (ECs) from local microvasculature and tumor cells secrete a wide range of enzymes that effectively

degrade the surrounding ECM to allow tumor cell invasion of the host tissue and microvascular ECs to migrate, proliferate and form a new blood supply to feed the growing tumor [7, 9]. A number of these enzymes have been discovered and characterized over the years such as metalloproteases, lysyl oxidases and cysteine and aspartyl cathepsins [7]. Interestingly, over the last couple of decades, aspartyl cathepsins, particularly cathepsin D (CathD), have gained increased attention due to their extracellular presence in the tumor microenvironment and reported roles in tumor development and metastasis as well as their potential as therapeutic targets [10-18].

CathD is a ubiquitous, aspartic endoproteinase that is expressed in all human tissues. Physiologically, it resides in the lysosomes, proteolytically degrading unfolded or non-functional proteins. CathD is involved in essential biological processes, such as during development and maintaining tissue homeostasis, where the enzyme is believed to act proteolytically outside its acidic milieu [19]. Thus, dysregulation of CathD expression and/or function is associated with pathologies such as atherosclerosis, neurological, dermatological disorders and cancer [20]. For instance, CathD secreted from tumor cells into the extracellular space has been suggested to play an important role in invasion and metastasis of breast cancer [21, 22]. Winiarski *et al.* also reported an overexpression and secretion of CathD in cancerous tissue and ascites of ovarian cancer patients [23], which enhanced proangiogenic responses such as proliferation, migration and angiogenic tube formation in local omental microvascular ECs [11]. Overexpression and hypersecretion of CathD have now been demonstrated in other cancer types including lung, prostate, endometrial, malignant glioma and melanoma, and the protein is considered to be a prognostic biomarker in breast cancer [24] and a potential marker in predicting prognosis of endometrial adenocarcinoma [25]. These data, along with unresolved complexity of the microenvironment which facilitates tumor cell invasion of local host tissue, highlights the importance of further research on the biological aspects and therapeutic purpose

of CathD in cancer development. This chapter focuses on cancer cell-secreted CathD in the tumor microenvironment and its role in tumor invasion, angiogenesis and metastasis, and also gives a brief perspective on the possibility of targeting extracellular CathD therapeutically.

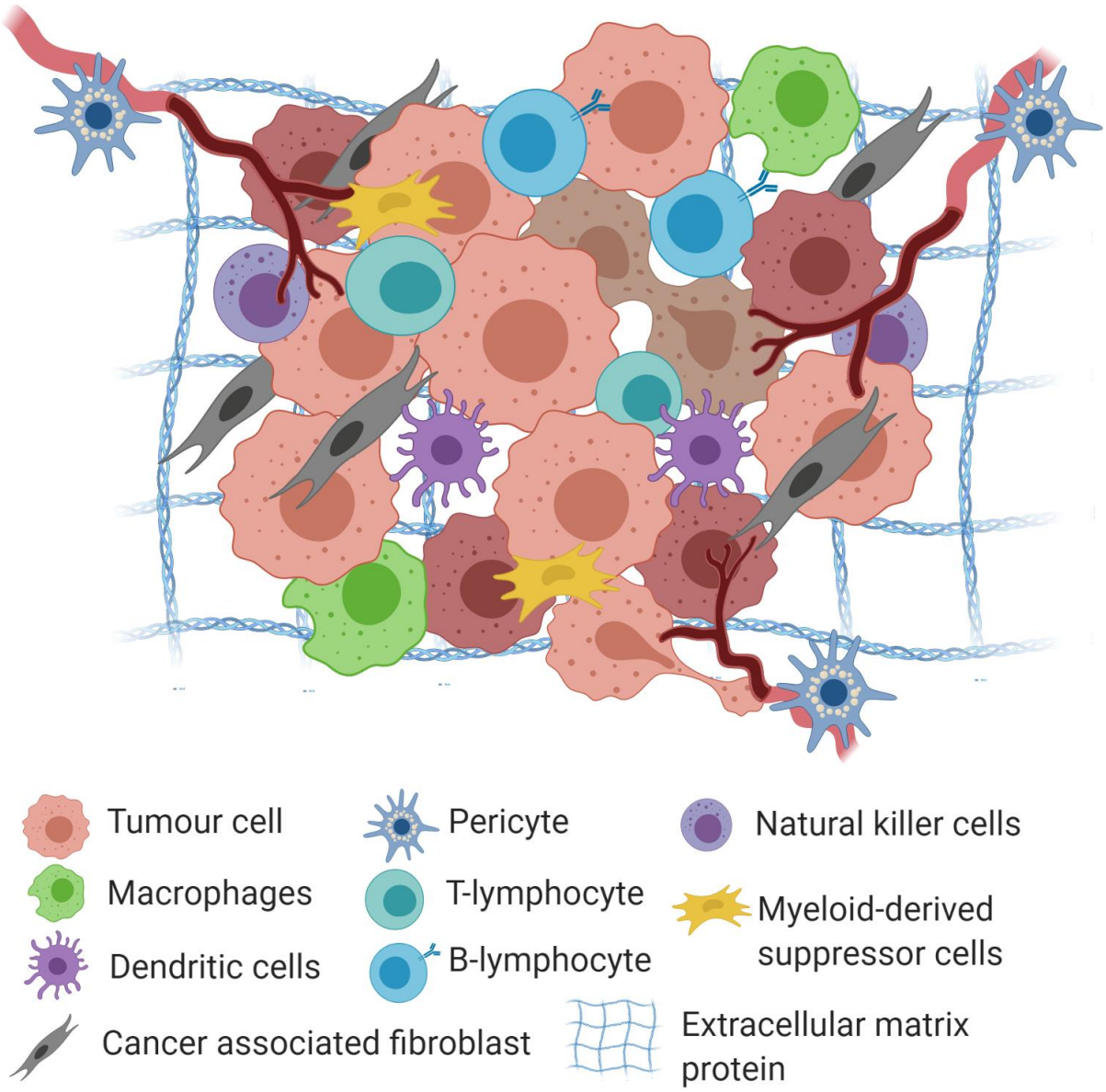

Figure 1: **Tumor microenvironment.** An illustration of the key cellular components of the tumor microenvironment.

Processing of Cathepsin D

The synthesis process of CathD is regulated in the conventional endoplasmic reticulum/Golgi pathway. After synthesis in the rough endoplasmic reticulum as inactive preprocathepsin D (43 kDa), it is further cleaved and glycosylated to form 52 kDa procathepsin D (pCathD) containing two N-linked oligosaccharides modified with mannose 6-phosphate (M6P) residues at asparagine residues 70 and 199 [26, 27]. Modified pCathD is then targeted to intracellular vesicular structures such as endosomes, lysosomes, and phagosomes both by M6P receptor (M6PR)-dependent and -independent pathways [19]. The latter mechanism of targeting is not yet understood; however, the sphingolipid activator precursor protein pro-saposin has been suggested to be involved [28].

Upon entry into the acidic milieu of the late endosome, M6PRs detach from pCathD and subsequently, the phosphate group is removed. Low pH- and cysteine protease-induced proteolytic cleavage of propeptide (44aa) of pCathD generates an active intermediate form of the enzyme [29]. The propeptide (also known as activation peptide) plays an essential role in correctly folding, activating and delivering the protein to lysosomes [30, 31]. This peptide, which is expressed in, and secreted from, cancer cells, has also been demonstrated to act as a growth factor for tumor cells [32]. The intermediate form of CathD is further cleaved to generate the mature form (48 kDa) containing a heavy chain (34 kDa) and a light chain (14 kDa) linked by non-covalent interactions [33]. CathD activity is tightly regulated at pH 3.5 [34], however, it is now known that the enzyme is active both proteolytically and non-proteolytically at neutral pH in the cytosol of apoptotic cells and during neurofibrillary degeneration and cancer progression [11, 15, 35, 36].

Physiological roles of CathD as both an intracellular and extracellular protein

Besides its lysosomal activity, CathD also plays a significant role during fetal development. There is a gradual maturation observed in the lysosomal system that correlates with increased CathD levels in all tissues [37]. Mice deficient in CathD survive during fetal development, but die around 1 month after birth due to significant neurodegeneration [38], indicating the protein's essential role in developmental biology. Further studies demonstrated that congenital mutations in the CathD gene lead to a reduction in expression and subsequent production of an enzymatically inactive protein that results in neurodegenerative disease in dogs and humans [39-44]. In a recent study, an association was shown between CathD deficiency and Parkinson's disease [45]. Interestingly, increased CathD expression and activity in cardiac cells is associated with heart failure in postpartum female mice [46]. Higher CathD levels also correlate with increasing apoptosis in the cerebellum and this has now been suggested to play a role in the pathogenesis of autism [47].

Other functions of CathD, related to its functional activity, have also been suggested. For instance, CathD-induced cleavage of metabolism-associated intracellular proteins, activation and degradation of polypeptide hormones and growth factors such as plasminogen, prolactin, endostatin, osteocalcin, thyroglobulin, insulin-like growth factor binding proteins (IGFBP) and secondary lymphoid tissue chemokine (SLC); activation of enzymatic precursors of CathL, CathB and transglutaminase 1; and processing of the enzyme activators and inhibitors prosaposin and cystatin C, reviewed in [19].

Although CathD mainly acts in the lysosome, in the last 2 decades its role in the extracellular space has been explored extensively. CathD differentiates from other aspartic endopeptidases in its packaging and sorting process. For instance, it has been known for a while that, physiologically, pCathD is sequestered to the lysosome and not secreted extracellularly. However, now we know that under some conditions, pCathD/CathD can escape the

conventional ER/Golgi-dependent targeting pathway and be secreted from cells. The most probable explanation is that over-expression of pCathD surpasses the limited number of M6PR binding sites available and thus, the protein accumulates in the cytosol and is subsequently secreted. The secretory mechanism, however, remains somewhat a mystery [48]. It is believed that the addition of carbohydrate groups to CathD during post-translational modification may determine its destiny [49]. For instance, tunicamycin, a glycosylation inhibitor, produced an unglycosylated form of CathD that was found to be secreted from cultured liver cells, suggesting that lysosomal enzyme-linked carbohydrate structures may play a crucial role in directing these enzymes [49]. In the case of secreted CathD, it is understood that these enzymes lack M6P residues, which is essential for sorting lysosomal enzymes. Different forms of CathD (or pCathD) are now known to be secreted in human, bovine and rat milk and serum, and the presence of both pCathD and CathD (34 kDa) was observed in human eccrine sweat and urine [50-53]. Interestingly, CathD in human eccrine sweat was found to be proteolytically active at sweat pH 5.5 [54], which agreed with the increasing evidence in pathologies such as cancer that extracellular CathD may act via its proteolytic-dependent mechanism.

Expression of CathD in cancer

CathD is now known to be a major secreted protein found in the cancer microenvironment. Over the last 2 decades, studies have shown increased overexpression and hypersecretion of CathD in numerous cancer types including ovarian cancer, breast cancer, endometrial cancer, lung cancer, malignant glioma, melanoma and prostate cancer (Table 1) [25, 55-68]. In breast cancer in particular, CathD is considered as a "marker" associated with metastasis. For instance, overexpression of CathD in breast cancer cells correlates with increased risk of clinical metastasis and short survival in breast cancer patients [56-58]. Interestingly, increased secreted levels of pCathD were also detected in the serum of patients with breast malignancy [69]. Another study revealed that the total concentration of CathD in breast cancer tissue was

much higher than in other tissues including normal mammary cells [70]. Additionally, Masson and colleagues showed, for the first time, that CathD expression is gradually increased as preadipocytes differentiate into mature adipocytes in both humans and mice [71]. CathD upregulation was also reported in obese subjects and mice, indicating a significant pro-adipogenesis role of CathD. Since adipocytes play a supportive role in the growth process of the breast, and as clinical studies have reported a role of obesity in the incidence of breast cancer, CathD upregulation may actually play an indirect role in breast cancer progression.

A role for CathD has now been shown in the progression of ovarian cancer metastasis. Earlier research investigating ovarian cancer suggested that the enhanced level of CathD expression was associated with increased cancer cell differentiation and with clinically advanced histological type [72, 73]. More recent studies have reported enhanced CathD expression as an indicator of malignancy in serous ovarian cancer [74-76], for instance, over 70% of invasive ovarian cancers were shown to express CathD [75]. Intriguingly, this finding was contradicted by another study which showed that high expression of CathD in the ovarian tumor was associated with a favorable survival prognosis [76]. However, our previous work investigating omental metastasis of ovarian cancer revealed that a high omental mesothelial expression of CathD (close to the metastatic tumor) was associated with poor disease-specific survival (DSS) [23]. The study also found that expression of CathD was significantly higher in the omental lesion of serous ovarian carcinoma compared with omentum from patients with benign ovarian cystadenoma [23], further supporting a potential pro-cancerous role of CathD in ovarian carcinoma.

Table 1. Involvement of CathD in the stages of tumor progression in different cancer types. Modified from [15]

Cancer Type	Metastasis	Invasion	Angiogenesis	References
Breast	↑	↑	↑	[56-59]
Ovarian	ND	ND	↑	[55, 11]
Prostate	↑	↑	↓	[60-62]
Endometrial	ND	↑	ND	[68]
Melanocytic	↑	↑	ND	[63]
Glioma	↑	↑	ND	[64]
Lung	ND	↑	ND	[67]

↑, increase in effects; ↓, reduction in effects; ND, not determined.

Role of CathD in tumor progression

Proteolytic-dependent roles

It is now becoming clear that CathD plays a role in the tumor microenvironment. However, a number of questions arise as to how this enzyme with an optimum pH of 3.5 acts proteolytically at neutral pHs. Earlier studies suggested that CathD plays an intracellular cytosolic role at neutral pH in inducing apoptosis, indicating its proteolytic capability at neutral or near-neutral pHs. The enzyme is translocated to the cytosol due to lysosomal membrane permeabilization and actively cleaves the BH3-interacting domain (Bid) to form truncated Bid (tBid) [36, 77, 78]. tBid activates the insertion of Bax into the mitochondrial membrane, leading to the release of cytochrome C from mitochondria into the cytosol [79-81]. This apoptotic response was

partially delayed by pepstatin A (pepA), an inhibitor of CathD proteolytic activity [78-80], suggesting a pro-apoptotic mechanism induced by this enzyme. The role of CathD in inducing *in vitro* apoptosis was further validated when a pan caspase inhibitor (Z-VAD-FMK) induced a significant reduction in cell death when given in combination with pepA [82, 83]. Additionally, tau protein degradation by cytosolic (i.e. pH 7) proteolytically active CathD has been reported in Alzheimer neurofibrillary degradation [35]. These studies strongly suggest that CathD is active at pHs higher than the optimum, although it should be noted that other works suggested that mutant CathD, deprived of its catalytic activity, was indistinguishable from that of the normal enzyme [84, 85].

Although it could be argued that a pro-apoptotic role for intracellular CathD may be anti-tumorigenic, this is in contrast to observations that indicate that not only is CathD secreted from tumor cells, but that this extracellular CathD may have key pro-tumorigenic functions. For instance, CathD was observed to be overexpressed and hyper-secreted from estrogen-positive MCF7 breast cancer cells in in *vitro* experiments, that resulted in enhanced tumor growth and invasion in mammary carcinogenesis [86]. Interestingly, CathD has been shown to cleave cell-secreted cystatin C, a potent endogenous inhibitor of cysteine and metalloproteinase, at a lower pH (pH 5.5- 6.8), similar to the *in vivo* tumor microenvironment [87]. This suggests that active CathD plays a significant role in tumor progression, by preventing the inhibitory action of cystatin C on proteases that actively cleave extracellular matrix protein in the tumor stroma, allowing cancer cells to invade local tissue. Interestingly, another study demonstrated that proteolytically active CathD stimulates the activity of secreted plasminogen activators by degrading plasminogen activator inhibitor-1 at pH 6.6 i.e. similar to the tumor microenvironment. The authors suggested that this process could be a contributory factor involved in triggering a proteolytic cascade facilitating breast cancer cell invasion and metastasis [88]. Intriguingly, CathD has also been shown to selectively degrade macrophage

inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4), and SLC (CCL21) that, in turn, may affect the generation of the anti-tumoral immune response, the migration of human breast cancer cells, or both processes [89].

Although secreted pCathD is generally considered to be proteolytically inactive [90-92], in the hypoxic, acidic tumor microenvironment, this precursor form of the enzyme may be converted, by an autocatalytic mechanism into the mature form capable of degrading ECM proteins, thus releasing basic fibroblast growth factor (bFGF) [10, 59, 93]. The combination of degradation of the ECM proteins and released bFGF **(Figure 2)**, a pro-proliferative growth factor, allows local tumor and ECs cells to grow and invade local host tissue, aiding tumor metastasis [94].

A more recent study has demonstrated that both pCathD and mature CathD are involved in the migration of mesenchymal stem cells (MSCs) to tumor sites [95]. MSCs are known to secrete cytokines and chemokines that trigger both pro- and anti-tumorigenic responses in the tumor microenvironment. CathD-induced homing of these stem cells to the tumor microenvironment facilitates a more aggressive invasion of tumor cells into the surrounding tissue [95]. The study further revealed that pCathD acted as a potent stimulator of MSC migration which was completely reversed in the presence of pepA. Further investigation revealed an interesting phenomenon whereby pCathD in the tumor microenvironment was suggested to be uptaken/endocytosed by MSCs and converted into a proteolytically active, mature form of CathD, which then induced migration and invasion of MSCs in the cancer stroma [95]. However, CathD or pCathD had no effect on cellular proliferation in this study, contradicting the previous reports.

Proteolytic-independent roles

To answer whether CathD acts in a non-proteolytic manner in the tumor microenvironment, a number of studies have been carried out. For example, pCathD has been reported to act as a mitogen i.e. a protein-ligand, rather than enzymatically, to stimulate MCF7 cell proliferation via an autocrine mechanism [96]. In recent years, numerous studies have emerged that suggest a non-proteolytic proangiogenic role for CathD both *in vivo* and *in vitro.* For instance, in xenografts (3Y-Ad12 cell line transfected with wild-type and/or mutated Asn 231 CathD) in an athymic mice model, overexpression of CathD correlated with increased vascular density. In these mice, a 1.5-fold and 1.9-fold increase in microvessel density was observed in the CathD and CathD-Asn 231 (proteolytically inactive; transfected mice) groups respectively, suggesting that CathD induces angiogenic effects via an unknown mechanism other than its proteolytic activity [14]. Another study reported that both pCathD and CathD induced proliferation and migration of breast cancer cells, fibroblasts and ECs in both a proteolytic dependent and independent manner [97].

A similar observation was made in epithelial ovarian cancer (EOC). In an investigation on potential non-VEGF pathways in inducing tumor angiogenesis, we implicated secreted factors such as CathD, CathL and IGFBP7 both *in vitro* and *in vivo* **(Table 2) [23, 55]**. For instance, high levels of CathD were found in the ascites of patients suffering from ovarian cancer (unpublished data) and CathD was later found to induce proangiogenic effects in disease-specific local microvascular endothelial cells [11, 55]. An increase in the secretion of CathD was also observed from EOC cancer cell lines (SKOV3 and A2780) [55], confirming the *in vivo* phenomenon. Our recent work demonstrated that exogenous CathD induces proliferation and migration of human omental microvascular ECs, suggesting a mitogenic role for this enzyme [11]. We further confirmed this proangiogenic response by showing activation of downstream signaling pathways (ERK1/2 and AKT) in response to CathD in these cells, which

agreed with a study where proteolytically inactive CathD was shown to induce human skin fibroblast proliferation via activation of the MAPK/ERK1/2 pathway [13] **(Figure 2)**. Interestingly, unlike previous observations, we found that CathD was not proteolytically active at neutral pHs, but highly active at low, acidic pHs (completely inhibited by pepA), suggesting that this enzyme acts non-proteolytically in the pre-tumor microenvironment of the secondary tumor site [11]. Our theory is that EOC-secreted CathD locally induces angiogenic responses i.e. EC proliferation and migration, during the initial stages of secondary tumor development i.e. in a pre-hypoxic, acidic environment. However, once secondary tumor foci are established in the omentum, CathD may act proteolytically in the tumor microenvironment to further accelerate the metastatic process, as indicated in the aforementioned studies. A similar observation was also observed for the EOC-secreted cysteine protease cathepsin L, whereby the enzyme non-proteolytically induced omental microvascular EC proliferation, although in this case the enzyme remained proteolytically active at neutral pHs [98].

Table 2: Proangiogenic factors secreted by ovarian cancer cells.

Activators	**Function**	**References**
Vascular endothelial growth factor (VEGF)	Stimulates angiogenesis, permeability	[99]
Cathepsin D	Stimulate EC proliferation and migration	[11]
Cathepsin L	Stimulate EC proliferation and migration	[98]
Angiopoietin-1 (Ang1) and Tie2 receptor	Ang1: stabilises vessels by strengthening endothelial-smooth muscle interactions; Tie2R: inhibits permeability	[100]
Fibroblast growth factor (FGF)	Stimulate angiogenesis and arteriogenesis	[101]
Transforming growth factor (TGF-β1)	Stabilises vessels by stimulating ECM production	[102]

Heparin-binding epidermal growth factor-like growth factor	Binds to epidermal growth factor receptor (EGFR) and promote angiogenesis	[103]
IL6	Induces migration of ECs in the mesentery in EOC	[102, 104]
IL8	Stimulates VEGF expression and the autocrine activation of VEGFR2 in ECs	[105, 102]

A proangiogenic role for CathD may be critical to its reported pro-tumorigenic importance and this has been explored in numerous other studies. For instance, CathD was found to induce blood vessel formation in the chick chorioallantoic membrane (CAM) model, [106]. A role for CathD in angiogenesis was further illustrated by the observation that migration of human umbilical vein ECs and *in vitro* angiogenic tube formation was increased when cells were treated with active pure CathD. The observation that pepA completely inhibited these effects manner indicated that CathD was proteolytically active in these experiments [106]. As mentioned previously, proteolytically active CathD has also been suggested to induce angiogenesis in breast cancer by cleaving and releasing ECM-bound pro-angiogenic bFGF [59]. The studies described support the suggestion that CathD can induce proangiogenic responses via both its proteolytic action and an unknown mechanism that is not dependent on its proteolytic activity.

In contrast, it has also been suggested that CathD activity may be anti-angiogenic. For instance, pCathD secreted by prostate cancer cells was shown to have a possible role in generating angiostatin via proteolysis—a specific inhibitor of angiogenesis *in vitro* as well as *in vivo* [62], suggesting an opposing effect of CathD in angiogenesis.

There is ample evidence that CathD may induce mitogenic responses in the cells of the tumor microenvironment via both proteolytic-dependent and independent mechanisms. Vignon *et al.* demonstrated that the precursor of CathD, pCathD, non-proteolytically induced growth of

MCF7 breast cancer cells *in vitro* [96]. A significant increase in human skin CCD45K fibroblast proliferation, motility, and invasive capacity was also observed to be induced by proteolytically active and inactive CathD [13]. This prompted an investigation into the target receptor molecule on these cells and the authors observed a partial reduction in fibroblast proliferation in the presence of M6P and pCathD. Further studies investigating the effects of CathD on tumor cells reported rapid growth of human CathD cDNA-transfected 3Y1-Ad12 rat tumor cells *in vitro,* with an increased experimental metastatic potential *in vivo* [107-109]. In addition, the proliferation of 3Y1-Ad12 cells was induced in response to both wild-type and mutated (Asn 231, proteolytically inactive) CathD *in vitro* and *in vivo* [12, 14]. Based on the previous study, the authors tested whether M6P inhibited CathD-induced proliferation and concluded that M6P did not compete with CathD interacting with M6PR, indicating a novel receptor, probably LDL receptor-related protein 1 (LRP1) [110], involved in inducing a cellular response. In the same study, the propeptide (27-44aa) of pCathD was found not to be mitogenic, contradicting studies which found otherwise [32, 70, 111-114].

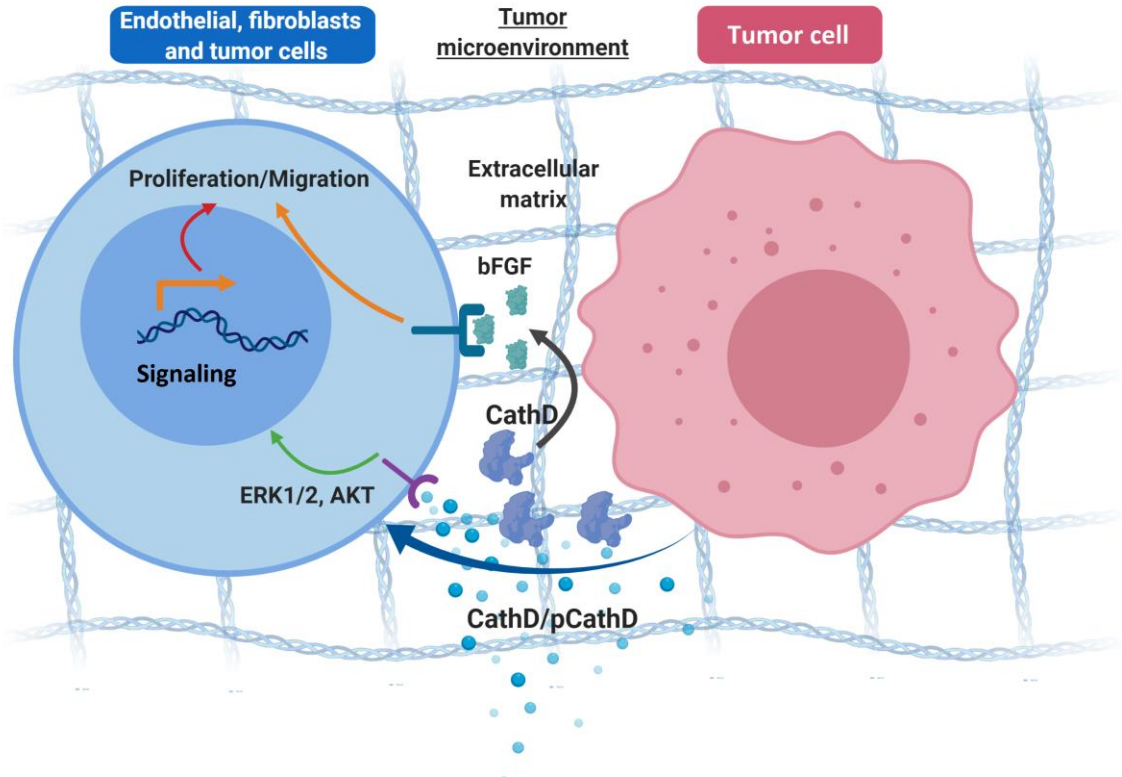

Figure 2. **Tumor cell-secreted CathD and its pro-cancerous role in the tumor microenvironment.** Overexpression of pCathD/CathD leads to its hypersecretion into the extracellular space by tumor cells. Proteolytically active CathD cleaves ECM proteins and releases the basic fibroblast growth factor (bFGF) that induces angiogenesis. Both pCathD and CathD induce tumor cell proliferation in a proteolytic-dependent and independent manner, thus utilizing an autocrine mechanism. CathD also induces proliferation of fibroblasts, and both proliferation and migration of ECs via activation of the ERK1/2 and AKT pathways.

Future perspective

As discussed above, the over-production and secretion of CathD could substantially contribute to tumor progression via directly influencing cancer cells and stromal cells such as fibroblasts and ECs non-proteolytically, and indirectly by cleaving ECM proteins, cytokines and chemokines locally. We recently showed that exogenous CathD promotes proliferation and migration in human omental microvascular ECs in ovarian cancer metastasis via inducing phosphorylation of the ERK1/2 and PI3K/AKT pathways in a proteolytic-independent manner [11], suggesting activation of a receptor tyrosine kinase. Recently, CathD was shown to induce

outgrowth of fibroblasts by binding to the LRP1 receptor which could potentially play a role in CAF proliferation in the tumor microenvironment, further aiding tumor growth [110]. A number of conventional anti-cancer strategies such as chemotherapy, radiotherapy and antiangiogenic therapy, are available to treat advanced disease. Importantly, antiangiogenic therapies such as anti-VEGF monoclonal antibody bevacizumab (Avastin) [115, 116], have been used clinically, but many have reported side-effects that limit safety in patients [117-120]. Recently, extracellular proteolytically active CathD was shown to play a pathogenic role in non-alcoholic steatohepatitis, particularly in regulating hepatic inflammation and dyslipidaemia [121], and it was demonstrated that inhibiting this enzyme protected mice from fatty liver disease [122]. Therefore, novel therapeutic targets, such as extracellular CathD, both in its proteolytic and non-proteolytic form, are urgently required.

Ashraf *et al.* recently demonstrated an anti-tumor efficacy for anti-CathD antibody in triple-negative breast cancer (TNBC) mice models [17]. TNBC, which accounts for 15-20% of all breast cancer cases, lacks overexpression of estrogen receptors, progesterone receptors and human epidermal growth factor receptor 2 (HER-2) [123]. Thus, the only available treatments are surgery, chemotherapy, and radiotherapy. Targeting extracellular CathD, which is overexpressed in TNBC [124], and is a strong marker for poor prognosis in breast cancer patients (with potent pro-tumorigenic effects) [11, 12, 24, 96, 125], via an immunotherapy approach could be of clinical significance. The authors in this recent study reported that two human anti-CathD antibodies efficiently bound to human and mouse CathD, even at the low pH of the TNBC microenvironment and significantly inhibited tumor growth in three different TNBC mouse models (MDA-MB-231 cell xenografts and two TNBC patient-derived xenografts) without apparent toxic effects [17]. Interestingly, the antibody prevented the recruitment of tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells within the tumor, which are known to play a role in tumor immunosuppression. In peritoneal

metastases, such as high-grade serous carcinoma (advanced ovarian cancer), TAMs constitute over 50% of cells in the peritoneal tumor implants and ascites [126]. CathD overexpression and hypersecretion are also observed in tumor-associated omental mesothelium and in ascites from patients [23] and ovarian tumor conditioned media [55], and CathD is now known to induce a proangiogenic effect in the tumor microenvironment [11]. Therefore, targeting CathD utilizing an immunotherapy approach may be safer and more efficacious in treating ovarian carcinoma. However, bioavailability, selective targeting, and drug-delivery pose greater challenges which would require further research.

Due to the complexity the tumor microenvironment presents, conventional drug delivery systems fail to deliver the chemotherapeutics at an effective concentration to selectively kill cancer cells and therefore can be associated with debilitating side effects. Thus, studies have been conducted to investigate alternative approaches to drug delivery such as utilizing nanotechnology. In recent years, nanomedicine and its underpinning sciences have significantly contributed to drug bioavailability and therapeutic index in cancer therapy. FDA approved nanostructures/chemo drugs such as liposomal formulation of doxorubicin (DOX) (Doxil® or Caelex®), daunorubicin (DaunoXome®) and albumin-bound paclitaxel (PTX) (Abraxane®) have been in use, however clinically, these formulations proved to be moderately successful due to inadequate delivery to the tumor microenvironment [127]. Therefore, in an attempt to target CathD, we developed a graphene-based compound (graphene oxide), that breaks down and adsorbs this protein [16]. Important characteristics of graphene oxide such as surface charge, large surface area, electronic features, chemical reactivity, and good bioavailability were utilized to entrap CathD *in vitro* [16]. Our data demonstrated that adsorption of CathD led to denaturation of the enzyme on the surface of graphene oxide. This promising outcome was also observed at low concentrations of graphene oxide, which remained non-toxic to cells *in vitro*. Thus, future work could address further development to

integrate targeted and safe delivery of graphene oxide to the tumor sites and testing of this compound in the tumor microenvironment *in vivo* tumor models, with a proven clearance of disseminated CathD and extracellular enzyme-targeting specificities.

Conclusions

The complexity of the tumor microenvironment such as the crosstalk between the cellular and non-cellular components, along with the barrier to drug delivery, poses greater challenges in discovering newer targets in cancer therapy. Conventional anti-cancer strategies have been the strongest weapons in defeating tumor, although, most of these fail to shrink tumors at secondary sites, limiting effective treatment. Current antiangiogenic therapies, in combination with chemotherapies moderately increase progression free survival, with side effects that could be life threatening. Therefore, newer targets within the microenvironment, such as extracellular CathD, which has a dual functionality, may hold greater promise in reducing breast and ovarian cancer progression.

References

1. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet. 2013;45(4):353-61. doi:10.1038/ng.2563.
2. Eccles SA, Aboagye EO, Ali S, Anderson AS, Armes J, Berditchevski F et al. Critical research gaps and translational priorities for the successful prevention and treatment of breast cancer. Breast Cancer Res. 2013;15(5):R92. doi:10.1186/bcr3493.
3. van Dam GM, Themelis G, Crane LM, Harlaar NJ, Pleijhuis RG, Kelder W et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-alpha targeting: first in-human results. Nat Med. 2011;17(10):1315-9. doi:10.1038/nm.2472.
4. Zugazagoitia J, Guedes C, Ponce S, Ferrer I, Molina-Pinelo S, Paz-Ares L. Current Challenges in Cancer Treatment. Clin Ther. 2016;38(7):1551-66. doi:10.1016/j.clinthera.2016.03.026.
5. Raave R, van Kuppevelt TH, Daamen WF. Chemotherapeutic drug delivery by tumoral extracellular matrix targeting. J Control Release. 2018;274:1-8. doi:10.1016/j.jconrel.2018.01.029.
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74. doi:10.1016/j.cell.2011.02.013.
7. De Palma M, Biziato D, Petrova TV. Microenvironmental regulation of tumour angiogenesis. Nat Rev Cancer. 2017;17(8):457-74. doi:10.1038/nrc.2017.51.

8. Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y et al. New horizons in tumor microenvironment biology: challenges and opportunities. BMC Med. 2015;13:45. doi:10.1186/s12916-015-0278-7.
9. Paiva AE, Lousado L, Guerra DAP, Azevedo PO, Sena IFG, Andreotti JP et al. Pericytes in the Premetastatic Niche. Cancer Res. 2018;78(11):2779-86. doi:10.1158/0008-5472.CAN-17-3883.
10. Briozzo P, Morisset M, Capony F, Rougeot C, Rochefort H. In vitro degradation of extracellular matrix with Mr 52,000 cathepsin D secreted by breast cancer cells. Cancer Res. 1988;48(13):3688-92.
11. Pranjol MZI, Gutowski NJ, Hannemann M, Whatmore JL. Cathepsin D non-proteolytically induces proliferation and migration in human omental microvascular endothelial cells via activation of the ERK1/2 and PI3K/AKT pathways. Biochim Biophys Acta Mol Cell Res. 2018;1865(1):25-33. doi:10.1016/j.bbamcr.2017.10.005.
12. Glondu M, Coopman P, Laurent-Matha V, Garcia M, Rochefort H, Liaudet-Coopman E. A mutated cathepsin-D devoid of its catalytic activity stimulates the growth of cancer cells. Oncogene. 2001;20(47):6920-9. doi:10.1038/sj.onc.1204843.
13. Laurent-Matha V, Maruani-Herrmann S, Prebois C, Beaujouin M, Glondu M, Noel A et al. Catalytically inactive human cathepsin D triggers fibroblast invasive growth. J Cell Biol. 2005;168(3):489-99. doi:10.1083/jcb.200403078.
14. Berchem G, Glondu M, Gleizes M, Brouillet JP, Vignon F, Garcia M et al. Cathepsin-D affects multiple tumor progression steps in vivo: proliferation, angiogenesis and apoptosis. Oncogene. 2002;21(38):5951-5. doi:10.1038/sj.onc.1205745.
15. Pranjol MZ, Gutowski N, Hannemann M, Whatmore J. The Potential Role of the Proteases Cathepsin D and Cathepsin L in the Progression and Metastasis of Epithelial Ovarian Cancer. Biomolecules. 2015;5(4):3260-79. doi:10.3390/biom5043260.
16. Tabish TA, Pranjol MZI, Horsell DW, Rahat AAM, Whatmore JL, Winyard PG et al. Graphene Oxide-Based Targeting of Extracellular Cathepsin D and Cathepsin L As A Novel Anti-Metastatic Enzyme Cancer Therapy. Cancers (Basel). 2019;11(3). doi:10.3390/cancers11030319.
17. Ashraf Y, Mansouri H, Laurent-Matha V, Alcaraz LB, Roger P, Guiu S et al. Immunotherapy of triple-negative breast cancer with cathepsin D-targeting antibodies. J Immunother Cancer. 2019;7(1):29. doi:10.1186/s40425-019-0498-z.
18. Dubey V, Luqman S. Cathepsin D as a Promising Target for the Discovery of Novel Anticancer Agents. Curr Cancer Drug Targets. 2017;17(5):404-22. doi:10.2174/1568009616666161229145115.
19. Benes P, Vetvicka V, Fusek M. Cathepsin D--many functions of one aspartic protease. Crit Rev Oncol Hematol. 2008;68(1):12-28. doi:10.1016/j.critrevonc.2008.02.008.
20. Cocchiaro P, De Pasquale V, Della Morte R, Tafuri S, Avallone L, Pizard A et al. The Multifaceted Role of the Lysosomal Protease Cathepsins in Kidney Disease. Front Cell Dev Biol. 2017;5:114. doi:10.3389/fcell.2017.00114.
21. Rochefort H. Biological and clinical significance of cathepsin D in breast cancer. Semin Cancer Biol. 1990;1(2):153-60.
22. Rochefort H. Cathepsin D in breast cancer. Breast Cancer Res Treat. 1990;16(1):3-13.
23. Winiarski BK, Cope N, Alexander M, Pilling LC, Warren S, Acheson N et al. Clinical Relevance of Increased Endothelial and Mesothelial Expression of Proangiogenic Proteases and VEGFA in the Omentum of Patients with Metastatic Ovarian High-Grade Serous Carcinoma. Transl Oncol. 2014;7(2):267-76 e4. doi:10.1016/j.tranon.2014.02.013.
24. Abbott DE, Margaryan NV, Jeruss JS, Khan S, Kaklamani V, Winchester DJ et al. Reevaluating cathepsin D as a biomarker for breast cancer: serum activity levels versus histopathology. Cancer Biol Ther. 2010;9(1):23-30. doi:10.4161/cbt.9.1.10378.

25. Losch A, Kohlberger P, Gitsch G, Kaider A, Breitenecker G, Kainz C. Lysosomal protease cathepsin D is a prognostic marker in endometrial cancer. British journal of cancer. 1996;73(12):1525-8.
26. Hasilik A, Klein U, Waheed A, Strecker G, von Figura K. Phosphorylated oligosaccharides in lysosomal enzymes: identification of alpha-N-acetylglucosamine(1)phospho(6)mannose diester groups. Proc Natl Acad Sci U S A. 1980;77(12):7074-8. doi:10.1073/pnas.77.12.7074.
27. Fortenberry SC, Schorey JS, Chirgwin JM. Role of glycosylation in the expression of human procathepsin D. J Cell Sci. 1995;108 (Pt 5):2001-6.
28. Gopalakrishnan MM, Grosch HW, Locatelli-Hoops S, Werth N, Smolenova E, Nettersheim M et al. Purified recombinant human prosaposin forms oligomers that bind procathepsin D and affect its autoactivation. The Biochemical journal. 2004;383(Pt. 3):507-15. doi:10.1042/BJ20040175.
29. Laurent-Matha V, Derocq D, Prebois 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. Journal of biochemistry. 2006;139(3):363-71. doi:10.1093/jb/mvj037.
30. Takeshima H, Sakaguchi M, Mihara K, Murakami K, Omura T. Intracellular targeting of lysosomal cathepsin D in COS cells. Journal of biochemistry. 1995;118(5):981-8.
31. Yasuda Y, Tsukuba T, Okamoto K, Kadowaki T, Yamamoto K. The role of the cathepsin E propeptide in correct folding, maturation and sorting to the endosome. Journal of biochemistry. 2005;138(5):621-30. doi:10.1093/jb/mvi159.
32. Vetvicka V, Vetvickova J, Fusek M. Role of procathepsin D activation peptide in prostate cancer growth. The Prostate. 2000;44(1):1-7.
33. Gieselmann V, Hasilik A, von Figura K. Processing of human cathepsin D in lysosomes in vitro. J Biol Chem. 1985;260(5):3215-20.
34. Yoshinari M, Taurog A. Lysosomal digestion of thyroglobulin: role of cathepsin D and thiol proteases. Endocrinology. 1985;117(4):1621-31. doi:10.1210/endo-117-4-1621.
35. Kenessey A, Nacharaju P, Ko LW, Yen SH. Degradation of tau by lysosomal enzyme cathepsin D: implication for Alzheimer neurofibrillary degeneration. Journal of neurochemistry. 1997;69(5):2026-38.
36. Roberg K, Johansson U, Ollinger K. Lysosomal release of cathepsin D precedes relocation of cytochrome c and loss of mitochondrial transmembrane potential during apoptosis induced by oxidative stress. Free radical biology & medicine. 1999;27(11-12):1228-37.
37. Kageyama T, Tatematsu M, Ichinose M, Yahagi N, Miki K, Moriyama A et al. Development-dependent expression of cathepsins d and e in various rat tissues, with special reference to the high expression of cathepsin e in fetal liver. Zoological science. 1998;15(4):517-23. doi:10.2108/0289-0003(1998)15[517:DEOCDA]2.0.CO;2.
38. Saftig P, Hetman M, Schmahl W, Weber K, Heine L, Mossmann H et al. Mice deficient for the lysosomal proteinase cathepsin D exhibit progressive atrophy of the intestinal mucosa and profound destruction of lymphoid cells. EMBO J. 1995;14(15):3599-608.
39. Tyynela J, Sohar I, Sleat DE, Gin RM, Donnelly RJ, Baumann M et al. A mutation in the ovine cathepsin D gene causes a congenital lysosomal storage disease with profound neurodegeneration. The EMBO journal. 2000;19(12):2786-92. doi:10.1093/emboj/19.12.2786.
40. Tyynela J, Sohar I, Sleat DE, Gin RM, Donnelly RJ, Baumann M et al. Congenital ovine neuronal ceroid lipofuscinosis--a cathepsin D deficiency with increased levels of the inactive enzyme. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2001;5 Suppl A:43-5.

41. Steinfeld R, Reinhardt K, Schreiber K, Hillebrand M, Kraetzner R, Bruck W et al. Cathepsin D deficiency is associated with a human neurodegenerative disorder. American journal of human genetics. 2006;78(6):988-98. doi:10.1086/504159.
42. Fritchie K, Siintola E, Armao D, Lehesjoki AE, Marino T, Powell C et al. Novel mutation and the first prenatal screening of cathepsin D deficiency (CLN10). Acta neuropathologica. 2009;117(2):201-8. doi:10.1007/s00401-008-0426-7.
43. Siintola E, Partanen S, Stromme P, Haapanen A, Haltia M, Maehlen J et al. Cathepsin D deficiency underlies congenital human neuronal ceroid-lipofuscinosis. Brain : a journal of neurology. 2006;129(Pt 6):1438-45. doi:10.1093/brain/awl107.
44. Awano T, Katz ML, O'Brien DP, Taylor JF, Evans J, Khan S et al. A mutation in the cathepsin D gene (CTSD) in American Bulldogs with neuronal ceroid lipofuscinosis. Molecular genetics and metabolism. 2006;87(4):341-8. doi:10.1016/j.ymgme.2005.11.005.
45. Cullen V, Lindfors M, Ng J, Paetau A, Swinton E, Kolodziej P et al. Cathepsin D expression level affects alpha-synuclein processing, aggregation, and toxicity in vivo. Molecular brain. 2009;2:5. doi:10.1186/1756-6606-2-5.
46. Hilfiker-Kleiner D, Kaminski K, Podewski E, Bonda T, Schaefer A, Sliwa K et al. A cathepsin D-cleaved 16 kDa form of prolactin mediates postpartum cardiomyopathy. Cell. 2007;128(3):589-600. doi:10.1016/j.cell.2006.12.036.
47. Sheikh AM, Li X, Wen G, Tauqeer Z, Brown WT, Malik M. Cathepsin D and apoptosis related proteins are elevated in the brain of autistic subjects. Neuroscience. 2010;165(2):363-70. doi:10.1016/j.neuroscience.2009.10.035.
48. Mathieu M, Vignon F, Capony F, Rochefort H. Estradiol down-regulates the mannose-6-phosphate/insulin-like growth factor-II receptor gene and induces cathepsin-D in breast cancer cells: a receptor saturation mechanism to increase the secretion of lysosomal proenzymes. Molecular endocrinology. 1991;5(6):815-22. doi:10.1210/mend-5-6-815.
49. Reithmeier RAF. Assembly of proteins into membranes. New Comprehensive Biochemistry. 1996: 425–71. doi: doi:10.1016/s0167-7306(08)60523-2.
50. Vetvicka V, Vagner J, Baudys M, Tang J, Foundling SI, Fusek M. Human breast milk contains procathepsin D--detection by specific antibodies. Biochem Mol Biol Int. 1993;30(5):921-8.
51. Larsen LB, Petersen TE. Identification of five molecular forms of cathepsin D in bovine milk. Adv Exp Med Biol. 1995;362:279-83.
52. Benes P, Koelsch G, Dvorak B, Fusek M, Vetvicka V. Detection of procathepsin D in rat milk. Comp Biochem Physiol B Biochem Mol Biol. 2002;133(1):113-8.
53. Zuhlsdorf M, Imort M, Hasilik A, von Figura K. Molecular forms of beta-hexosaminidase and cathepsin D in serum and urine of healthy subjects and patients with elevated activity of lysosomal enzymes. Biochem J. 1983;213(3):733-40.
54. Baechle D, Flad T, Cansier A, Steffen H, Schittek B, Tolson J et al. Cathepsin D is present in human eccrine sweat and involved in the postsecretory processing of the antimicrobial peptide DCD-1L. J Biol Chem. 2006;281(9):5406-15. doi:10.1074/jbc.M504670200.
55. Winiarski BK, Wolanska KI, Rai S, Ahmed T, Acheson N, Gutowski NJ et al. Epithelial ovarian cancer-induced angiogenic phenotype of human omental microvascular endothelial cells may occur independently of VEGF signaling. Transl Oncol. 2013;6(6):703-14.
56. Rochefort H. Cathepsin D in breast cancer: a tissue marker associated with metastasis. European journal of cancer. 1992;28A(11):1780-3.
57. Ferrandina G, Scambia G, Bardelli F, Benedetti Panici P, Mancuso S, Messori A. Relationship between cathepsin-D content and disease-free survival in node-negative breast cancer patients: a meta-analysis. British journal of cancer. 1997;76(5):661-6.

58. Foekens JA, Look MP, Bolt-de Vries J, Meijer-van Gelder ME, van Putten WL, Klijn JG. Cathepsin-D in primary breast cancer: prognostic evaluation involving 2810 patients. British journal of cancer. 1999;79(2):300-7. doi:10.1038/sj.bjc.6690048.
59. Briozzo P, Badet J, Capony F, Pieri I, Montcourrier P, Barritault D et al. MCF7 mammary cancer cells respond to bFGF and internalize it following its release from extracellular matrix: a permissive role of cathepsin D. Experimental cell research. 1991;194(2):252-9.
60. Chen L, Li H, Liu W, Zhu J, Zhao X, Wright E et al. Olfactomedin 4 suppresses prostate cancer cell growth and metastasis via negative interaction with cathepsin D and SDF-1. Carcinogenesis. 2011;32(7):986-94. doi:10.1093/carcin/bgr065.
61. Konno S, Cherry JP, Mordente JA, Chapman JR, Choudhury MS, Mallouh C et al. Role of cathepsin D in prostatic cancer cell growth and its regulation by brefeldin A. World journal of urology. 2001;19(4):234-9.
62. Morikawa W, Yamamoto K, Ishikawa S, Takemoto S, Ono M, Fukushi J et al. Angiostatin generation by cathepsin D secreted by human prostate carcinoma cells. J Biol Chem. 2000;275(49):38912-20. doi:10.1074/jbc.M005402200.
63. Zhu L, Wada M, Usagawa Y, Yasukochi Y, Yokoyama A, Wada N et al. Overexpression of cathepsin D in malignant melanoma. Fukuoka igaku zasshi = Hukuoka acta medica. 2013;104(10):370-5.
64. Fukuda ME, Iwadate Y, Machida T, Hiwasa T, Nimura Y, Nagai Y et al. Cathepsin D is a potential serum marker for poor prognosis in glioma patients. Cancer Res. 2005;65(12):5190-4. doi:10.1158/0008-5472.CAN-04-4134.
65. Rochefort H, Garcia M, Glondu M, Laurent V, Liaudet E, Rey JM et al. Cathepsin D in breast cancer: mechanisms and clinical applications, a 1999 overview. Clinica chimica acta; international journal of clinical chemistry. 2000;291(2):157-70.
66. Pruitt FL, He Y, Franco OE, Jiang M, Cates JM, Hayward SW. Cathepsin D acts as an essential mediator to promote malignancy of benign prostatic epithelium. The Prostate. 2013;73(5):476-88. doi:10.1002/pros.22589.
67. Vetvicka V, Vetvickova J, Benes P. Role of enzymatically inactive procathepsin D in lung cancer. Anticancer Res. 2004;24(5A):2739-43.
68. Nazeer T, Malfetano JH, Rosano TG, Ross JS. Correlation of tumor cytosol cathepsin D with differentiation and invasiveness of endometrial adenocarcinoma. American journal of clinical pathology. 1992;97(6):764-9.
69. Brouillet JP, Dufour F, Lemamy G, Garcia M, Schlup N, Grenier J et al. Increased cathepsin D level in the serum of patients with metastatic breast carcinoma detected with a specific pro-cathepsin D immunoassay. Cancer. 1997;79(11):2132-6.
70. Vetvicka V, Vektvickova J, Fusek M. Effect of human procathepsin D on proliferation of human cell lines. Cancer Lett. 1994;79(2):131-5.
71. Masson O, Prebois C, Derocq D, Meulle A, Dray C, Daviaud D et al. Cathepsin-D, a key protease in breast cancer, is up-regulated in obese mouse and human adipose tissue, and controls adipogenesis. PLoS One. 2011;6(2):e16452. doi:10.1371/journal.pone.0016452.
72. Baekelandt M, Holm R, Trope CG, Nesland JM, Kristensen GB. The significance of metastasis-related factors cathepsin-D and nm23 in advanced ovarian cancer. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 1999;10(11):1335-41.
73. Ferrandina G, Scambia G, Fagotti A, D'Agostino G, Benedetti Panici P, Carbone A et al. Immunoradiometric and immunohistochemical analysis of Cathepsin D in ovarian cancer: lack of association with clinical outcome. British journal of cancer. 1998;78(12):1645-52.
74. Henzen-Logmans SC, Fieret EJ, Berns EM, van der Burg ME, Klijn JG, Foekens JA. Ki-67 staining in benign, borderline, malignant primary and metastatic ovarian tumors:

correlation with steroid receptors, epidermal-growth-factor receptor and cathepsin D. International journal of cancer Journal international du cancer. 1994;57(4):468-72.
75. Losch A, Schindl M, Kohlberger P, Lahodny J, Breitenecker G, Horvat R et al. Cathepsin D in ovarian cancer: prognostic value and correlation with p53 expression and microvessel density. Gynecol Oncol. 2004;92(2):545-52. doi:10.1016/j.ygyno.2003.11.016.
76. Chai Y, Wu W, Zhou C, Zhou J. The potential prognostic value of cathepsin D protein in serous ovarian cancer. Archives of gynecology and obstetrics. 2012;286(2):465-71. doi:10.1007/s00404-012-2318-2.
77. Ollinger K. Inhibition of cathepsin D prevents free-radical-induced apoptosis in rat cardiomyocytes. Archives of biochemistry and biophysics. 2000;373(2):346-51. doi:10.1006/abbi.1999.1567.
78. Kagedal K, Johansson U, Ollinger K. The lysosomal protease cathepsin D mediates apoptosis induced by oxidative stress. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2001;15(9):1592-4.
79. Heinrich M, Neumeyer J, Jakob M, Hallas C, Tchikov V, Winoto-Morbach S et al. Cathepsin D links TNF-induced acid sphingomyelinase to Bid-mediated caspase-9 and -3 activation. Cell death and differentiation. 2004;11(5):550-63. doi:10.1038/sj.cdd.4401382.
80. Blomgran R, Zheng L, Stendahl O. Cathepsin-cleaved Bid promotes apoptosis in human neutrophils via oxidative stress-induced lysosomal membrane permeabilization. Journal of leukocyte biology. 2007;81(5):1213-23. doi:10.1189/jlb.0506359.
81. Johansson AC, Steen H, Ollinger K, Roberg K. Cathepsin D mediates cytochrome c release and caspase activation in human fibroblast apoptosis induced by staurosporine. Cell death and differentiation. 2003;10(11):1253-9. doi:10.1038/sj.cdd.4401290.
82. Zuzarte-Luis V, Montero JA, Torre-Perez N, Garcia-Porrero JA, Hurle JM. Cathepsin D gene expression outlines the areas of physiological cell death during embryonic development. Developmental dynamics : an official publication of the American Association of Anatomists. 2007;236(3):880-5. doi:10.1002/dvdy.21076.
83. Zuzarte-Luis V, Montero JA, Kawakami Y, Izpisua-Belmonte JC, Hurle JM. Lysosomal cathepsins in embryonic programmed cell death. Developmental biology. 2007;301(1):205-17. doi:10.1016/j.ydbio.2006.08.008.
84. Beaujouin M, Baghdiguian S, Glondu-Lassis M, Berchem G, Liaudet-Coopman E. Overexpression of both catalytically active and -inactive cathepsin D by cancer cells enhances apoptosis-dependent chemo-sensitivity. Oncogene. 2006;25(13):1967-73. doi:10.1038/sj.onc.1209221.
85. Tardy C, Tyynela J, Hasilik A, Levade T, Andrieu-Abadie N. Stress-induced apoptosis is impaired in cells with a lysosomal targeting defect but is not affected in cells synthesizing a catalytically inactive cathepsin D. Cell death and differentiation. 2003;10(9):1090-100. doi:10.1038/sj.cdd.4401272.
86. Rochefort H, Capony F, Garcia M, Cavailles V, Freiss G, Chambon M et al. Estrogen-induced lysosomal proteases secreted by breast cancer cells: a role in carcinogenesis? J Cell Biochem. 1987;35(1):17-29. doi:10.1002/jcb.240350103.
87. Laurent-Matha V, Huesgen PF, Masson O, Derocq D, Prebois C, Gary-Bobo M et al. Proteolysis of cystatin C by cathepsin D in the breast cancer microenvironment. FASEB J. 2012;26(12):5172-81. doi:10.1096/fj.12-205229.
88. Maynadier M, Farnoud R, Lamy PJ, Laurent-Matha V, Garcia M, Rochefort H. Cathepsin D stimulates the activities of secreted plasminogen activators in the breast cancer acidic environment. Int J Oncol. 2013;43(5):1683-90. doi:10.3892/ijo.2013.2095.
89. Wolf M, Clark-Lewis I, Buri C, Langen H, Lis M, Mazzucchelli L. Cathepsin D specifically cleaves the chemokines macrophage inflammatory protein-1 alpha, macrophage inflammatory protein-1 beta, and SLC that are expressed in human breast cancer. Am J Pathol. 2003;162(4):1183-90.

90. Rochefort H, Capony F, Garcia M. Cathepsin D: a protease involved in breast cancer metastasis. Cancer Metastasis Rev. 1990;9(4):321-31.
91. Capony F, Rougeot C, Montcourrier P, Cavailles V, Salazar G, Rochefort H. Increased secretion, altered processing, and glycosylation of pro-cathepsin D in human mammary cancer cells. Cancer Res. 1989;49(14):3904-9.
92. Richo G, Conner GE. Proteolytic activation of human procathepsin D. Adv Exp Med Biol. 1991;306:289-96. doi:10.1007/978-1-4684-6012-4_35.
93. Westley BR, May FE. Cathepsin D and breast cancer. Eur J Cancer. 1996;32A(1):15-24.
94. Crowe DL, Shuler CF. Regulation of tumor cell invasion by extracellular matrix. Histology and histopathology. 1999;14(2):665-71.
95. Vangala G, Imhoff FM, Squires CML, Cridge AG, Baird SK. Mesenchymal stem cell homing towards cancer cells is increased by enzyme activity of cathepsin D. Exp Cell Res. 2019. doi:10.1016/j.yexcr.2019.07.007.
96. Vignon F, Capony F, Chambon M, Freiss G, Garcia M, Rochefort H. Autocrine growth stimulation of the MCF 7 breast cancer cells by the estrogen-regulated 52 K protein. Endocrinology. 1986;118(4):1537-45. doi:10.1210/endo-118-4-1537.
97. Ohri SS, Vashishta A, Proctor M, Fusek M, Vetvicka V. The propeptide of cathepsin D increases proliferation, invasion and metastasis of breast cancer cells. International journal of oncology. 2008;32(2):491-8.
98. Pranjol MZI, Gutowski NJ, Hannemann M, Whatmore JL. Cathepsin L Induces Proangiogenic Changes in Human Omental Microvascular Endothelial Cells via Activation of the ERK1/2 Pathway. Curr Cancer Drug Targets. 2019;19(3):231-42. doi:10.2174/1568009618666180831123951.
99. Banerjee S, Kaye S. The role of targeted therapy in ovarian cancer. Eur J Cancer. 2011;47 Suppl 3:S116-30. doi:10.1016/S0959-8049(11)70155-1.
100. Lin Z, Liu Y, Sun Y, He X. Expression of Ets-1, Ang-2 and maspin in ovarian cancer and their role in tumor angiogenesis. J Exp Clin Cancer Res. 2011;30:31. doi:10.1186/1756-9966-30-31.
101. Tebben PJ, Kalli KR, Cliby WA, Hartmann LC, Grande JP, Singh RJ et al. Elevated fibroblast growth factor 23 in women with malignant ovarian tumors. Mayo Clin Proc. 2005;80(6):745-51. doi:10.1016/S0025-6196(11)61528-0.
102. Toutirais O, Chartier P, Dubois D, Bouet F, Leveque J, Catros-Quemener V et al. Constitutive expression of TGF-beta1, interleukin-6 and interleukin-8 by tumor cells as a major component of immune escape in human ovarian carcinoma. European cytokine network. 2003;14(4):246-55.
103. Tanaka Y, Miyamoto S, Suzuki SO, Oki E, Yagi H, Sonoda K et al. Clinical significance of heparin-binding epidermal growth factor-like growth factor and a disintegrin and metalloprotease 17 expression in human ovarian cancer. Clin Cancer Res. 2005;11(13):4783-92. doi:10.1158/1078-0432.CCR-04-1426.
104. Nilsson MB, Langley RR, Fidler IJ. Interleukin-6, secreted by human ovarian carcinoma cells, is a potent proangiogenic cytokine. Cancer Res. 2005;65(23):10794-800. doi:10.1158/0008-5472.CAN-05-0623.
105. Lokshin AE, Winans M, Landsittel D, Marrangoni AM, Velikokhatnaya L, Modugno F et al. Circulating IL-8 and anti-IL-8 autoantibody in patients with ovarian cancer. Gynecol Oncol. 2006;102(2):244-51. doi:10.1016/j.ygyno.2005.12.011.
106. Hu L, Roth JM, Brooks P, Luty J, Karpatkin S. Thrombin up-regulates cathepsin D which enhances angiogenesis, growth, and metastasis. Cancer Res. 2008;68(12):4666-73. doi:10.1158/0008-5472.CAN-07-6276.
107. Garcia M, Derocq D, Pujol P, Rochefort H. Overexpression of transfected cathepsin D in transformed cells increases their malignant phenotype and metastatic potency. Oncogene. 1990;5(12):1809-14.

108. Liaudet E, Garcia M, Rochefort H. Cathepsin D maturation and its stimulatory effect on metastasis are prevented by addition of KDEL retention signal. Oncogene. 1994;9(4):1145-54.
109. Liaudet E, Derocq D, Rochefort H, Garcia M. Transfected cathepsin D stimulates high density cancer cell growth by inactivating secreted growth inhibitors. Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research. 1995;6(9):1045-52.
110. Derocq D, Prebois C, Beaujouin M, Laurent-Matha V, Pattingre S, Smith GK et al. Cathepsin D is partly endocytosed by the LRP1 receptor and inhibits LRP1-regulated intramembrane proteolysis. Oncogene. 2012;31(26):3202-12. doi:10.1038/onc.2011.501.
111. Fusek M, Vetvicka V. Mitogenic function of human procathepsin D: the role of the propeptide. The Biochemical journal. 1994;303 (Pt 3):775-80.
112. Vetvicka V, Vetvickova J, Fusek M. Effect of procathepsin D and its activation peptide on prostate cancer cells. Cancer Lett. 1998;129(1):55-9.
113. Vetvicka V, Vetvickova J, Fusek M. Anti-human procathepsin D activation peptide antibodies inhibit breast cancer development. Breast cancer research and treatment. 1999;57(3):261-9.
114. Vetvicka V, Vetvickova J, Hilgert I, Voburka Z, Fusek M. Analysis of the interaction of procathepsin D activation peptide with breast cancer cells. International journal of cancer Journal international du cancer. 1997;73(3):403-9.
115. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med. 2011;365(26):2484-96. doi:10.1056/NEJMoa1103799.
116. Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol. 2012;30(17):2039-45. doi:10.1200/JCO.2012.42.0505.
117. Stone RL, Sood AK, Coleman RL. Collateral damage: toxic effects of targeted antiangiogenic therapies in ovarian cancer. Lancet Oncol. 2010;11(5):465-75. doi:10.1016/S1470-2045(09)70362-6.
118. Rodriguez M. Ziv-aflibercept use in metastatic colorectal cancer. J Adv Pract Oncol. 2013;4(5):348-52.
119. Scappaticci FA, Skillings JR, Holden SN, Gerber HP, Miller K, Kabbinavar F et al. Arterial thromboembolic events in patients with metastatic carcinoma treated with chemotherapy and bevacizumab. J Natl Cancer Inst. 2007;99(16):1232-9. doi:10.1093/jnci/djm086.
120. Tateo S, Mereu L, Salamano S, Klersy C, Barone M, Spyropoulos AC et al. Ovarian cancer and venous thromboembolic risk. Gynecol Oncol. 2005;99(1):119-25. doi:10.1016/j.ygyno.2005.05.009.
121. Houben T, Oligschlaeger Y, Hendrikx T, Bitorina AV, Walenbergh SMA, van Gorp PJ et al. Cathepsin D regulates lipid metabolism in murine steatohepatitis. Sci Rep. 2017;7(1):3494. doi:10.1038/s41598-017-03796-5.
122. Khurana P, Yadati T, Goyal S, Dolas A, Houben T, Oligschlaeger Y et al. Inhibiting Extracellular Cathepsin D Reduces Hepatic Steatosis in Sprague(-)Dawley Rats (dagger). Biomolecules. 2019;9(5). doi:10.3390/biom9050171.
123. O'Toole SA, Beith JM, Millar EK, West R, McLean A, Cazet A et al. Therapeutic targets in triple negative breast cancer. J Clin Pathol. 2013;66(6):530-42. doi:10.1136/jclinpath-2012-201361.

124. Huang L, Liu Z, Chen S, Liu Y, Shao Z. A prognostic model for triple-negative breast cancer patients based on node status, cathepsin-D and Ki-67 index. PLoS One. 2013;8(12):e83081. doi:10.1371/journal.pone.0083081.
125. Vetvicka V, Benes P, Fusek M. Procathepsin D in breast cancer: what do we know? Effects of ribozymes and other inhibitors. Cancer Gene Ther. 2002;9(10):854-63. doi:10.1038/sj.cgt.7700508.
126. Gupta V, Yull F, Khabele D. Bipolar Tumor-Associated Macrophages in Ovarian Cancer as Targets for Therapy. Cancers (Basel). 2018;10(10). doi:10.3390/cancers10100366.
127. Fernandes C, Suares D, Yergeri MC. Tumor Microenvironment Targeted Nanotherapy. Front Pharmacol. 2018;9:1230. doi:10.3389/fphar.2018.01230.