REVIEW

Involvement of cathepsins in the invasion, metastasis and proliferation of cancer cells

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Abstract : Tumor cell invasion and metastasis are associated with the proteolytic activity of various types of proteinases. Among them, cathepsins, which are lysosomal proteinases, have received more attention recently. Since elevated expressions of cathepsins and diminished levels of their inhibitors have been observed in several human cancers, including breast, gastric and prostate cancer, especially in aggressive cancer cells, cathepsins have been suggested to be biological markers of malignant tumors and have proved useful for prognosis of the disease. Furthermore, cathepsins have various roles in cancer progression. Cathepsin D has a mitogenic activity independent of its proteolytic activity and it attenuates the anti-tumor immune response of decaying chemokines to inhibit the function of dendritic cells. Cathepsins B and L have been shown to play an important role in matrix degradation and cell invasion. The administration of their inhibitors prevents the invasion and metastasis of cancer cells. These results indicate that cancer cells orchestrate various cathepsins to progress malignant diseases. Cathepsins may be a potential target for cancer therapy. J. Med. Invest. 52 : 1-9, February, 2005

Keywords **:** *cancer, cathepsins, inhibitors, invasion, metastasis*

INTRODUCTIOIN

Tumor invasion and metastasis are responsible for the progression of malignant diseases. These processes are facilitated by the up-regulation of various types of proteinases, which induce the escape of cancer cells from the primary site, breaking down connective barriers of the extracellular matrix and basement membrane (1, 2). In addition to metallo and serine proteinases, such as MMP-1 or MMP-9 well known to be secreted outside cells, there is increasing evidence that lysosomal proteinase, cathepsin B, L and D, may also play an important role in the development and progression of malignant tumors (3-6).

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Cathepsins B, L and D are lysosomal cysteine and aspartic proteinases, distributed in almost all mammalian cells. They undergo post-translational modification of mannose-6 - phosphate residues and are translocated to lysosomal vesicles via the receptor, which is the main pathway of intracellular protein turnover (7). The overexpression of cathepsin D (Cath-D) in aggressive cancer is observed in breast cancer and prostate cancer, and is associated with a poor prognosis (4, 8 -10). The expression of this proteinase is regulated by estrogens in breast cancer cells through a non-consensual estrogen-responsive element (11, 12). In some cancer cells, cathepsins B (Cath-B) and L (Cath-L) are either secreted or associated with the plasma membrane (13-15) and degrade the extracellular matrix during tumor progression. Their activities are regulated by endogenous inhibitors, cystatins A and B (Cys. A, Cys. B), and cystatin C. The imbalance between proteases and inhibitors is also an important factor in deciding if the cancer is

malignant (16).

The purpose of this review is to provide an overview of various new roles of cathepsins in cancer, considering their potency as a therapeutic target.

CATH-D IN CANCER

Cath-D has mitogenic activity

In breast tumors, Cath-D has been suggested to act as a mitogen, promoting tumor growth through the enzymatically inactive pro-peptide (17). The exogenous addition of Cath-D in MCF-7 breast cancer cells showed mitogenic activity (18). Glondu and colleagues reported that the down-regulation of Cath-D expression by antisense gene transfer inhibited Matrigel outgrowth and experimental lung metastasis in human MDA-MB-231 breast cancer cells (19). Cath-D is indicated to be more essential for tumor growth than for extracellular matrix invasion because the effect of Cath-D on cancer cell proliferation and tumor angiogenesis is independent of its proteolytic activity. Wild-type Cath-D and its mutant were expressed in 3Y1-Ad 12 rat tumor cells (21). Wild-type Cath-D, as well as its mutated form lacking proteolytic activity, stimulated tumor growth. However, only the wild type inhibited tumor apoptosis, whereas the inactive form did not. Therefore, Cath-D might provide some protection against tumor apoptosis by proteolysis (19, 20). A synthetic peptide of the precursor domain of Cath-D showed no mitogenic effect, suggesting that a receptor of the pro-fragment was not involved. Furthermore, the mitogenic activity of Cath-D was not blocked by inhibiting the interaction of pro-cath-D with mannose-6-phosphate receptors (21). An excess of Man-6-P partially inhibited pro-cath-D binding and endocytosis, indicating the presence of a Man-6-P independent receptor for endocytosis that might be related to Cath-D mitogenic activity. These results suggest that Cath-D acts as a protein ligand interacting with a mitogenic receptor. However the mechanism of mitogenic activity is still unclear.

Cath-D attenuates anti-tumoral immune response

Purified Cath-D, as well as culture supernatants from the human breast carcinoma cell lines, MCF-7 and T 47D, selectively digests macrophage inflammatory protein (MIP)-1α, MIP-1β, and secondary lymphoid-tissue chemokine (SLC) (Table 1) (22). The proteolytic activity was most efficient at pH 4.0 and could not be detected at a pH greater than 5.5. Such an acidic condition may be rare in the extracellular environment of normal tissues. However, it is well known that the extracellular pH of tumors is acidic (23). Breast cancer cells and macrophages within malignant tissue have high potential to release protons into the extracellular milieu through the proton pump of vacuolar H^+-ATPase at the plasma membrane (24). V-ATPase is involved in tumor cell invasion (25). Sennoune and colleagues revealed that V-ATPase was located at the plasma membrane of human breast cancer cells and its activity was greater in highly metastatic cells than in slightly metastatic cells (26). V-ATPase inhibitors attenuated the invasion or migration in highly metastatic cells.

Table 1. Chemokine Processing by Cathepsin D (Respective Receptors in Brackets)

Processed by Cath-D	Not processed by Cath-D
MIP-1α (CCR1, CCR5)	IL-6, GCP-2 (CXCR 1,CXCR 2)
MIP-1β (CCR 5)	Mig, IP-10, I-TAC (CXCR3)
SLC (CCR 7)	SDF-1(CXCR4)
	BCA-1(CXCR5)
	RANTES (CCR1, CCR3, CCR5)
	MCP-1(CCR2)
	MCP-2(CCR3, CCR5)
	MCP-3(CCR1, CCR2, CCR3)
	MCP-4(CCR2, CCR3)
	Eotaxin, Eotaxin-2 (CCR3)
	ELC (CCR 7)
	I-309(CCR 8)

(Wolf *et al*., 2003)

MIP-1α and MIP-1β, which are targets of Cat-D digestion, are implicated in the migration of immature dendritic cells (DCs) that express the CC chemokine receptor (CCR)1[receptor of RANTES (regulated upon activation, normal T cell expressed and secreted) and MIP-1 α] and CCR5[receptor of RANTES and MIP-1 α/β] in tumor tissues (27). Furthermore, immature DCs mature and up-regulate the expression of the SLC receptor CCR7 after exposure to neoplastic cells or tumor stroma (28), and initiate a tumoricidal immune response at the primary sites (29). Antigen-loaded mature DCs can then promote an anti-tumor effect by migrating to secondary lymphoid organs (30). As Wolf and colleagues (22) hypothesized, the cleavage of SLC by Cath-D within primary tumor sites may interfere with the migration of mature DCs to secondary lymphoid organs, representing an escape mechanism of the anti-tumoral immune response. These data suggest that Cath-D attenuates the anti-tumoral immune response by degradation of the chemokines that activate and migrate DCs (Fig.1). Several chemokines, not cleaved by Cath-D (such as RANTES [CCL 5],

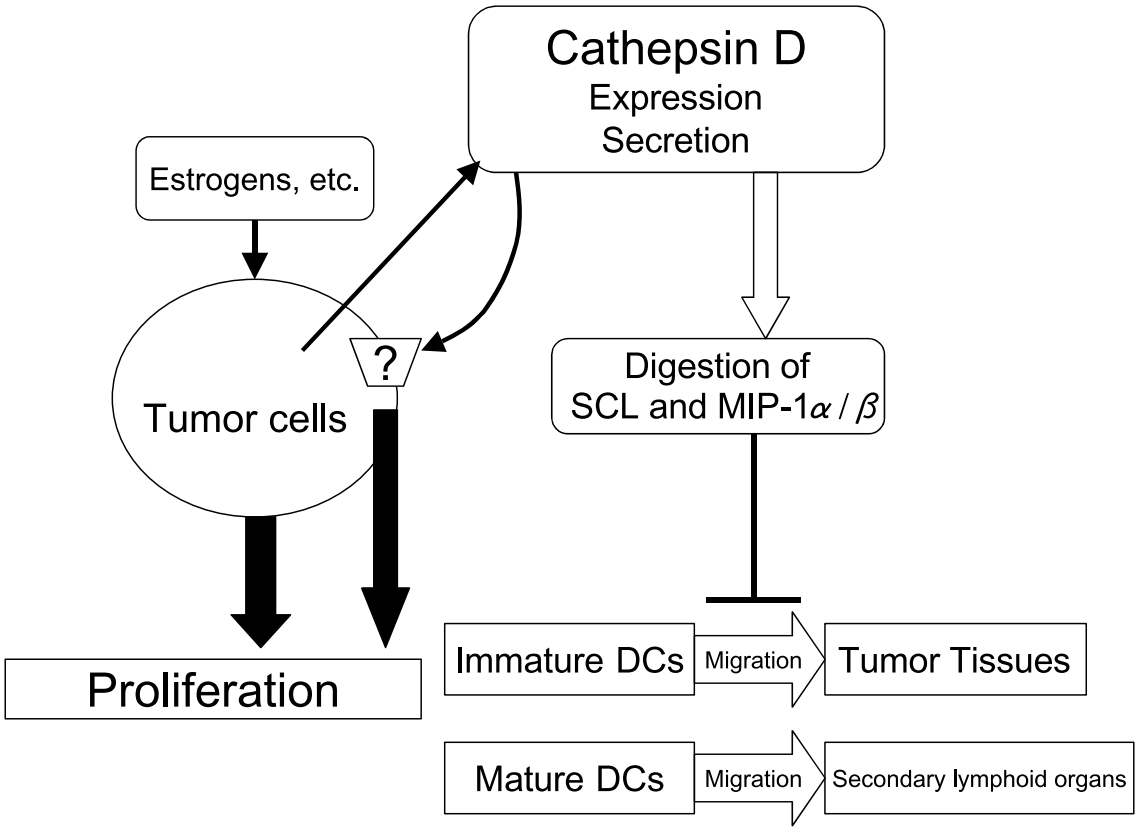

Fig.1. Cath-D has a dual effect, stimulating tumor cell proliferation and inhibiting dendritic cells (DCs) migration. Estrogens stimulate cancer cells for the proliferation and expression of Cath-D. Secreted Cath-D promotes tumor growth autocrinely and digests SLC and MIP-1 α/β. SLC and MIP-1 α/β regulate the migration of dendritic cells promoting an anti-tumor effect.

MCP-1 [CCL2], SDF-1 [stromal cell-derived factor-1], and IL-8), are highly expressed in breast cancers (31-34). SDF-1 is thought to promote the motility of cancer cells and regulates the directional migration of breast cancer cells to sites of metastasis (35). The chemokine-proteinase network may regulate cancer progression in the tumoral extracellular microenvironment.

EXPRESSION OF CATHS-B AND L PARTICIPATES IN MALIGNANCY

Recently, evidences have shown that the activity of Cath-B and L increases in breast cancer (5, 36, 37), prostate cancer (38) and in early stage gastric carcinoma (39). Cath-B and L were shown to be markers for malignant breast and prostate tumors and were suggested as useful prognostic factors for disease relapse, because this expression correlated with the invasiveness of breast and prostate carcinomas (38, 40-42). The enzyme activity increased in highly tumorigenic lines compared to poorly tumorigenic lines.

IMBALANCE BETWEEN CATHS AND INHIBITORS

Cathepsin activity and cysteine proteinase inhibitors are thought to play an important role in the processes of tumor invasion and metastasis (43), and the imbalance between them causes invasion and metastasis in cancer cells (Fig.2) (16).

Significantly reduced levels of cysteine proteinase inhibitor were observed in malignant prostate tissue samples (38) and prostate (44) and breast (42) tumor cell lines. Stefin A (StA) protein decreased with invasiveness and reported tumorigenicity, and Cys. B protein was significantly lower in all MDA-MB lines compared with the least invasive and tumorigenic MCF7 line (42). The imbalance contributes to the development of a malignant cell phenotype (3). Prostate carcinoma with an aggressive clone had a greater ratio of Cath-B to Cys. A (45). Less aggressive clones had a lower ratio. Sinha and colleagues showed a significant positive association between the ratio of Cath-B to Cys. A a nd the incidence of pelvic lymph node metastasis.

Cath-B and L activity increased significantly in DU 145-conditioned media at high cell density (44). The ratio of cathepsin activity to cycteine proteinase inhibi-

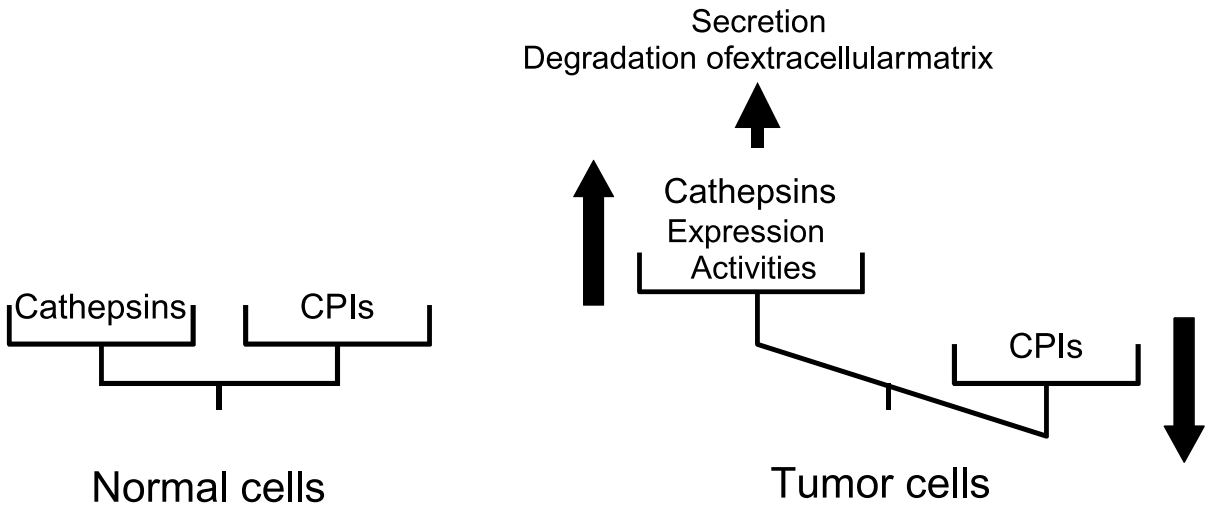

Fig.2. The imbalance between Caths and cystein protease inhibitors (CPIs) in tumor cells. Overexpression and secretion of cathepsins induce the invasion and metastasis of tumor cells.

tors increased in DU 145 and PC3 cell cultures with increasing density. DU145 and PC3 penetrated the Matrigel® matrix and this invasion increased with increasing cell density. The ability of cystatin-transfected PC 3 cells to invade the Matrigel® was attenuated compared to non-transfected cells or cells transfected with the vector alone (46).

INHIBITION OF CATHEPSINS IN CANCER CELLS

Antisense studies of Cath-B and L

Mohanam and colleagues transfected human glioblastma cells SNB19 with a plasmid containing Cath-B cDNA in antisense orientation (47). Clones expressing antisense Cath-B cDNA exhibited significant reductions in Cath-B mRNA and enzyme activity. A Matrigel® invasion assay showed that the antisense-transfected cells had markedly diminished invasiveness compared with the controls. An intracerebral injection of SNB 19 stable antisense transfectants resulted in reduced tumor formation in nude mice. Szpaderska and Frankfater also transfected human melanoma A375M and human prostate carcinoma PC3M with a plasmid containing Cath-B cDNA in antisense orientation (48). These cells produced 40-50% less Cath-B than control cells and were proportionately less invasive. Similarly, the inhibition of Cath-L overexpression in murine myeloma cells by an antisense approach reduced their tumorigenic potential (49). These cell lines with low Cath-L activity exhibited a significant reduction of tumorigenesis in nude mice, when compared with control cells expressing wild-type levels of Cath-L activity.

These results strongly supported the important role of Cath-B and L in the invasiveness of cancer cells and suggested cysteine proteinase inhibitors may be good candidates for cancer therapy.

Inhibitor studies of Cath-B and L

Chicken cystatin, a commonly used natural inhibitor of cysteine proteinases, effectively suppressed the invasion of ras-transformed human breast epithelial cells, MCF-10 A neoT (50). Cystatin had no effect on cell viability. Recently, various specific inhibitors for individual cathepsins have been synthesized (51). It has also been reported that Cath-B and L inhibitors prevent cancer cell invasion, cancer metastasis and cancer-induced osteoporosis. CA-074, a specific inhibitor of Cath-B, was developed as the epoxysuccinyl peptides by Katunuma's group (52-54). Specific inhibitors for cathepsin L were developed by Katunuma *et al*. and Yasuma *et al*. (55, 56).

CA-074 decreased the Matrigel® invasiveness of prostate cancer-cell PC 3 M at a concentration of 10 μM (48) although it had an opposite effect at 1μM. CA-074 equally neutralized extracellular Cath-B activity at 1 and 10 μM, and 1 μM of CA-074 only weakly inhibited intracellular Cath-B activity. CA-074 Me, a membrane-permeant pro-inhibitor which converts to CA-074 after internalization (57), completely abolished intracellular Cath-B activity and produced a 45-75% inhibition of invasion (48). Bervar and colleagues also reported that membrane permeability was very important for a Cath-B inhibitor to show an inhibitory effect on the invasion of the human breast cancer cell, MCF10AT(58). These results suggested that Cath-B had an important intracellular function in matrix degradation and invasion.

On the other hand, E-64, a non-selective cysteine

proteinase inhibitor, reduced invasion but had no effect on intracellular Cath-B activity(50). E-64 had no effect on cell motility. One or more cysteine proteinases, except cathepsin B, may be involved in matrix degradation.

CLIK-148, a specific Cath-L inhibitor, was evaluated for protection against bone metastasis of cancer cells (16). Colon 26 murine carcinoma PMF-15cells were subcutaneously inoculated over the calvaria of CDF1 mice to induce calvaria adsorption. The calvarial calcium contents in tumor-bearing calvarias decreased to about 30% of that in normal calvarias, and *iv* or po administration of CLIK-148 significantly suppressed the decreases

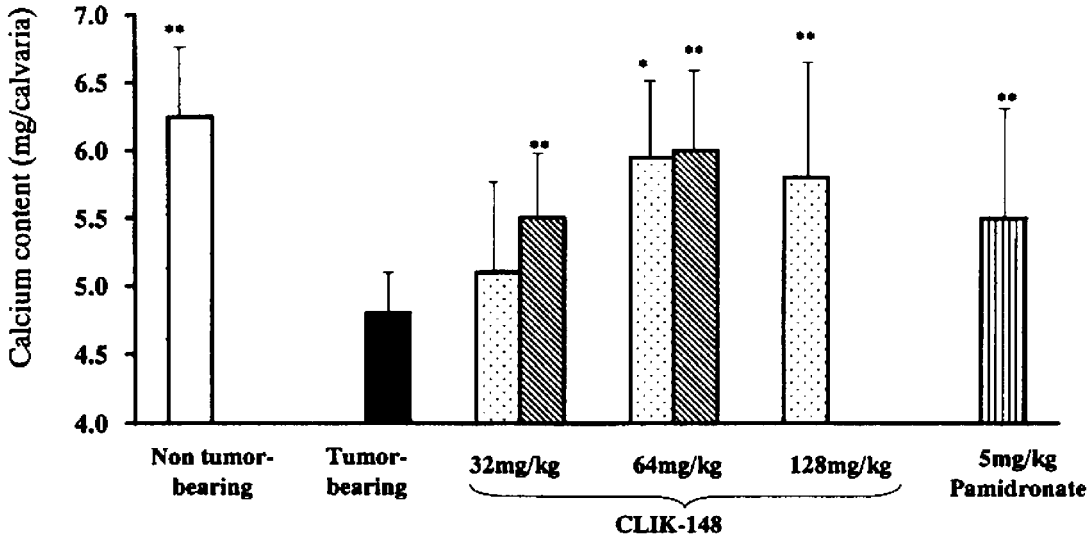

Fig.3. Inhibitory effect of mouse calvaria bone absorption induced by tumor implantation using CLIK-148. Colon tumor 26 PMF-15 cells (1×10^6cells/mice) were subcutaneously inoculated over the calvaria of CDF1 mice on day 0. CLIK-148 was administered po (⊡) or *iv* (▨) from days 1 to 7. A vehicle was administered orally in the non-tumor bearing (■) and tumor-bearing control groups (□). Pamidronate (▥, 5 mg/kg) was administered by i.v. on days 1, 3, and 5 as a positive control. On day 8, the calcium content of the calvaria was determined spectroscopically. Values are the means and SD (n=8). $^{**}P<0.01$ vs. tumor-bearing control by Welch's t-test.

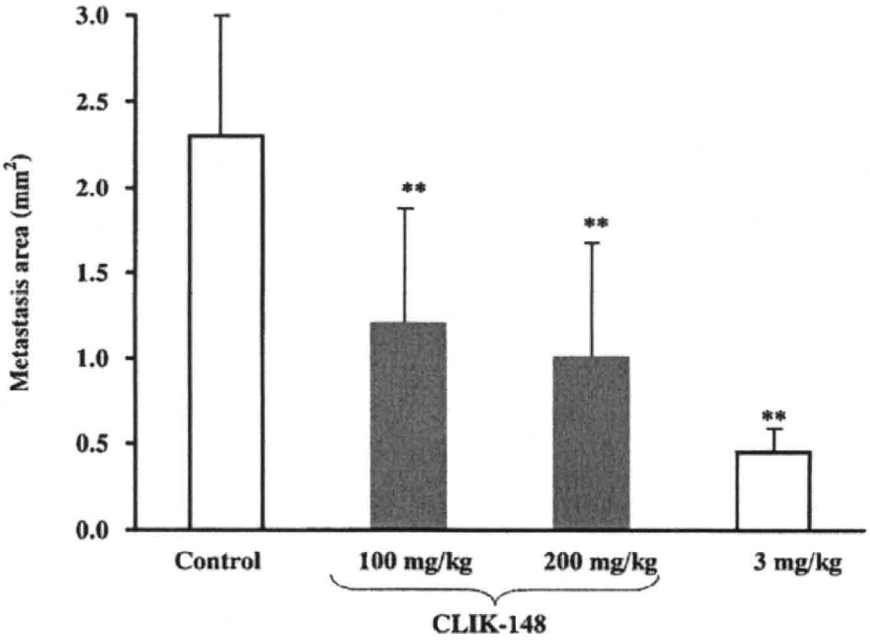

Fig.4. Suppression by CLIK-148 of distant bone metastasis created by the implantation of human melanoma A375 cells. A375 cells (1×10^6 cells/mice) were injected into the left ventricle of a mouse heart on day 0. CLIK-CLIK-148 (100, 200 mg/kg) was administered from day 7 to 20 (po, bid), and incadronate (3 mg/kg) was administered on days 7 and 14 (iv) as a positive control. The values are the means and SD of the area of bone metastasis. $^{**}P<0.01$ vs. tumor-bearing control by Welch's t-test.

in the level of calvarial calcium (Fig. 3) (16). CLIK-148 treatment also reduced the distant bone metastasis model of human melanoma A375 cells, which were implanted into the left ventricle of the heart and invaded via systemic circulation (Fig.4) (16). Bone metastasis is very common in prostate cancer and is responsible for the morbidity of this disease (59, 60). The Cath-L inhibitor has the potential for treatment.

Joyce and colleagues showed that JPM-OEt, an analog of irreversible Cath binding scaffold E-64 as a broad-spectrum cysteine proteinase inhibitor (61), had profound effects on tumor growth, invasiveness, and angiogenic switching, disrupting both early and late stages of tumorigenesis in RIP1-Tag2 transgenic mice (6). They found that the expression and activity of several Caths were upregulated in the invasive edges of pancreatic islet carcinomas during RIP1-Tag2 tumorigenesis. In contrast, both individual gene knockouts of members of the MMP and serine proteinase family (MMP-2, MMP-9 and uPA) and broad-spectrum MMP inhibition did not affect to the progression of invasive carcinomas in this model (62). They propose that Caths facilitate invasive growth capability, through the proteolysis of E-cadherin. The loss of E-cadherin function is a determinant of the invasive growth capability in this transgenic mouse (63).

CONCLUSION

The role of Caths in cancer progression is summarized in Table 2. Cath-D has mitogenic activity in cancer cells and reduces the primary immune response inhibiting the functions of chemokines. Cath-B works intracellularly and results in cancer cell in invasion or matrix degradation. Cath-L has an important role in extracellular matrix degradation. In cancer tissues, these Caths may act in concert to promote malignancy and can therefore be a good target for cancer therapy.

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Table 2. Role of cathepsin D, B and L in tumor

	Mitogenic activity on cancer cells	Proteinase activity	Inhibitory effect
Cathepsin D	+	Degradation of MIP-1α/β, SLC	Anti-tumoral immune response
Cathepsin B	-	Intracellular function in matrix degradation	Blockade of invasion and metastasis
Cathepsin L	-	Extracellular function in matrix degradation	Blockade of invasion and metastasis

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