

Expression of Cathepsin L in Human Tumors

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ABSTRACT

It has been proposed that proteases secreted by cancer cells facilitate tumor invasion and metastasis by degrading the components of extracellular membranes. The lysosomal cysteine protease cathepsin L is synthesized in large amounts and secreted by many malignantly transformed cells in culture. The secreted protease is potent in degrading collagen, laminin, elastin, and other structural proteins of basement membranes. To determine whether human cancers synthesize cathepsin L, the expression of cathepsin L in approximately 100 human tumor samples was determined by quantitative RNA slot blot analysis using a specific human cathepsin L complementary DNA probe. Results of the present study suggest that cancers in general express higher levels of cathepsin L than do normal tissues. Kidney and testicular tumors expressed the highest levels of cathepsin L; non-small cell carcinomas of the lung expressed the next highest levels; and most cancers of the breast, ovary, colon, adrenal, bladder, prostate, and thyroid expressed elevated levels as well. Cathepsin L may prove useful as a diagnostic or prognostic marker of human malignancy.

INTRODUCTION

Cathepsin L is a lysosomal acid cysteine protease present in all cell types where it is presumed to be primarily responsible for the degradation and turnover of intracellular proteins (1). Malignantly transformed mouse fibroblasts synthesize and secrete large amounts of cathepsin L, also known as MEP² (2). Its precise role in malignant transformation is not known, but it is a marker of the transformed state in several tissue culture systems (2-4). Human cathepsin L has been cloned and sequenced, indicating that human cathepsin L is encoded by a 1.6- to 1.8-kilobase mRNA which has 70% sequence homology to mouse cathepsin L (5, 6). Cathepsin L of both mice and humans is synthesized as proenzymes which are processed to the *M_r* 41,000 proenzymes (for humans) or *M_r* 38,000 proenzymes (for mice) which are secreted by many cells (7). Although the primary secreted form of these enzymes is probably inactive, it is rapidly autoactivated to lower molecular weight active forms similar to these found in lysosomes (8, 9).

Several hypotheses regarding the function of secreted cathepsin L in normal physiology and in malignancy have been suggested (10). Roles in antigen presentation (11), bone resorption (12), and sperm maturation (13) have been shown. The large amount of cathepsin L synthesized by mouse fibroblasts upon malignant transformation suggests that it may modulate the levels of proteins directly or indirectly involved in the growth regulation of these cells. Increased levels of secreted cathepsin L, taken together with its ability to degrade collagen, elastin, laminin, and other components of the extracellular matrix *in vitro*, suggest that cathepsin L may play a role in tumor invasion or metastasis *in vivo*.

The association of cathepsin L with malignant transforma-

tion in the mouse is known (2, 3, 14, 15). However, information on expression of this protease in human carcinoma is limited to two tissues (16, 17). To study the association of cathepsin L expression with malignancy in human tissues, we measured RNA levels in approximately 100 cancer specimens. The results demonstrate elevated expression of cathepsin L in many different cancers.

MATERIALS AND METHODS

Probes. Our laboratory previously cloned a human cathepsin L cDNA (pHu16) by screening a cDNA library made from human fibroblast RNA using mouse cathepsin L cDNA as probe (5). An 800-base pair *EcoRI* fragment was excised from the middle of pHu16 and purified on low-melting-point agarose. This fragment was radiolabeled by nick translation immediately before use as a probe. A 2.1-kilobase *XhoI* fragment of human β -actin cDNA was processed the same way and used as a control probe (18). Mouse RNA blots were hybridized with a full-length mouse cathepsin L cDNA (19) and normalized with the 1.15-kilobase *PstI* fragment of a mouse β -actin cDNA (20).

Cell Culture. Human KB epidermal carcinoma cells (a HeLa variant) were obtained from American Type Culture Collection and subcloned twice in the laboratory as described before (21). The KB 3-1 cells used in the present study are a clone of those cells. The cells were grown as monolayers in Dulbecco's modified Eagle's medium with 10% fetal calf serum, glutamine, penicillin (50 units/ml), and streptomycin (50 units/ml). The cells used for the isolation of RNA were 70 to 80% confluent.

RNA Isolation and Slot Blot Analysis. The processing and isolation of total cellular RNA from tumor tissues were as described earlier (22). Total cellular RNA from cultured cells was extracted by the single step method of Chomczynski and Sacchi (23). After extraction and quantitation, the RNA from various untreated tumors, shown to be intact by 0.8% agarose gel electrophoresis, was blotted on nitrocellulose filters. On each blot KB 3-1 RNA, which gave a reproducible cathepsin L signal, was blotted as a reference standard.

The nitrocellulose filter presoaked in 10 \times SSC was assembled in the slot blot apparatus as per Schleicher and Schuell instructions. Various amounts (10, 3, 1.0, and 0.3 μ g) of total RNA were diluted in a final volume of 100 μ l in 10 \times SSC and denatured by heating at 68 $^{\circ}$ C for 10 min before blotting. RNA was cross-linked to the nitrocellulose filters with a UV Stratalinker. The blots were prehybridized overnight at 42 $^{\circ}$ C in 50% formamide, 5 \times Denhardt's solution, 0.1% SDS, 100 μ g/ml of sheared salmon sperm DNA, and 5 \times SSC. Simultaneously, a radiolabeled probe was prehybridized against control pGEM3 plasmid blotted on a nitrocellulose filter in the above solution containing 10% dextran sulfate. After 16 h this solution containing the radiolabeled probe was used for hybridization of the RNA blot at 42 $^{\circ}$ C for 24 h. Then the blot was washed with 2 \times SSC-0.2% SDS at room temperature for 10 min followed by two washes with 0.1 \times SSC-0.1% SDS at 60 $^{\circ}$ C for 30 min each. The blots were exposed to Kodak XOMat film with intensifying screens at -70 $^{\circ}$ C for various periods.

Northern Blotting. The Northern blot analysis was performed as described by Thomas (24) with a slight modification. An aliquot containing 10 μ g of RNA was heated to 65 $^{\circ}$ C in 50% formamide, 6% formaldehyde, and 1 \times electrophoresis buffer (20 mM morpholinopropanesulfonic acid, 5 mM sodium acetate, and 1 mM EDTA, pH 7.0) for 10 min, immediately chilled on ice, and loaded on a 1.4% agarose gel containing 2.2 M formaldehyde and 1 \times electrophoresis buffer. The gel was run overnight at 50 V, and the RNA was transferred to a 0.22- μ m pore size nitrocellulose filter with 10 \times SSC for 20 h. The RNA was cross-linked to nitrocellulose and processed similarly as described for the slot blots.

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² The abbreviations used are: MEP, major excreted protein; cDNA, complementary DNA; SSC, standard saline citrate; SDS, sodium dodecyl sulfate.

Table 1 Levels of cathepsin L mRNA in normal mouse and human tissues

Values for the various human tissues for cathepsin L RNA levels were assigned by comparison with KB 3-1 cathepsin L mRNA levels which were given a value of 10 arbitrary units per 10 µg of RNA. In mouse cells and tissues, NIH 3T3 RNA was used as a similar internal reference standard for comparing different blots. Mouse liver, the organ with the highest expression of cathepsin L in the mouse, was assigned a value equal to that found in human liver, and all other mouse tissues are expressed relative to this value.

Organ	Human	Mouse
Brain	ND ^a	1.1
Heart	ND	2.8
Intestine	3.0	0.2
Kidney	5.0	5.6
Liver	7.0	7.0
Lung	6.0	1.4
Spleen	ND	1.4
Stomach	2.0	1.4

^a ND, not done.

RESULTS

The levels of cathepsin L RNA in many human cancers and several normal tissues were quantitated by slot blot analysis. KB 3-1 RNA was included on every blot and used as a reference standard for comparing the cathepsin L RNA levels of tumors on different blots. Cathepsin L message levels so determined were normalized using β-actin mRNA expression as an internal standard to control for variability in RNA quality. Table 1 describes the distribution of cathepsin L RNA in various normal human and mouse tissues. The expression of cathepsin L in single samples of various human tissues in descending order was as follows: liver > lung > kidney > adrenal = colon = intestine = stomach > esophagus. Due to the limited availability of normal human tissues, the levels of cathepsin L RNA in various mouse tissues were also estimated, and the results are shown in Table 1. The pattern of the cathepsin L mRNA distribution in various mouse tissues was similar to that observed in humans except for lung, which displayed higher levels than kidney in humans, but in the mouse, levels in lung were less than those in kidney.

Levels of cathepsin L mRNA in various cancers were also measured by slot blot analysis. Serial dilutions of RNA on blots displayed proportionately decreasing intensity of the signal on autoradiograms. Cathepsin L mRNA was normalized for equal loading of RNA by β-actin message. A typical slot blot analysis is shown in Fig. 1.

The levels of cathepsin L mRNA in various human cancers are summarized in Fig. 2. Kidney and testis tumors were found to express the highest levels of cathepsin L among the types of tumors studied. The average mRNA level for cathepsin L in

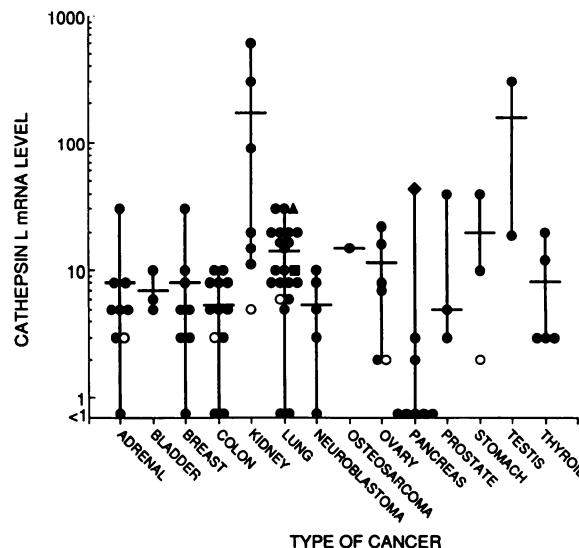
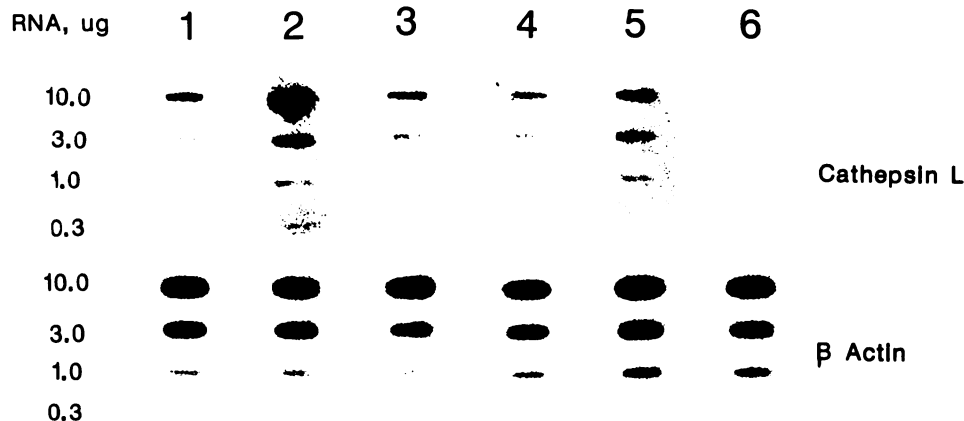


Fig. 2. Levels of cathepsin L RNA in various human cancers. RNA levels were estimated by slot blot analysis as described in "Materials and Methods" and the legend to Fig. 1. The cathepsin L signal obtained from 10 µg of KB 3-1 total RNA was assigned an arbitrary value of 10. KB 3-1 RNA was blotted on every blot and thus served as reference for comparing the cathepsin L mRNA in different tumors. O, normal tissue; ●, tumors; ■, small cell carcinoma of the lung; ▲, large cell carcinoma of the lung; ◆, pancreatic adenocarcinoma.

ovary, stomach, lung, colon, and adrenal tumors was severalfold higher than for normal tissues (Fig. 2). Most of the lung cancers were non-large cell in origin, but a single large cell cancer examined did express high levels of cathepsin L mRNA, with somewhat lower levels in a single small cell carcinoma. Similarly, in the pancreas the levels of cathepsin L RNA were severalfold higher in a single adenocarcinoma as compared with islet cell and other endocrine tumors which are usually benign. Levels of cathepsin L mRNA in breast, bladder, thyroid, and prostate tumors were also high, but we had no normal tissues with which to compare these values. Very little mRNA for cathepsin L was detected in Wilms' tumors, fibrosarcomas, leukemia, ganglioneuroma, and lymphoma specimens (data not shown).

Northern blot analysis for some tumor RNAs was performed to confirm the size of the message that was detected by the human cathepsin L cDNA probe on the slot blots. The 800-base pair EcoRI fragment from the full-length cDNA of human cathepsin L detected a single 1.8-kilobase band in each case (Fig. 3). The same size mRNA was detected in the KB-3-1 cell RNA sample which was used as reference for comparing the

Fig. 1. RNA slot blot analysis of human tumors. Total RNA isolated from various tumors was diluted serially (10.0, 3.0, 1.0, and 0.3 µg) in 10× SSC and then blotted onto nitrocellulose filters. KB 3-1 RNA was also blotted to serve as a standard for comparison. The blots were hybridized with the human cathepsin L probe, washed, and subjected to autoradiography (top). The cathepsin L probe was then removed, and the same blots were hybridized with the human β-actin probe to confirm the equal loading of RNA from different tumors (bottom). Lane 1, KB 3-1; Lane 2, epidermoid lung cancer; Lane 3, osteosarcoma; Lane 4, neuroblastoma; Lane 5, breast cancer; Lane 6, lymphoma.



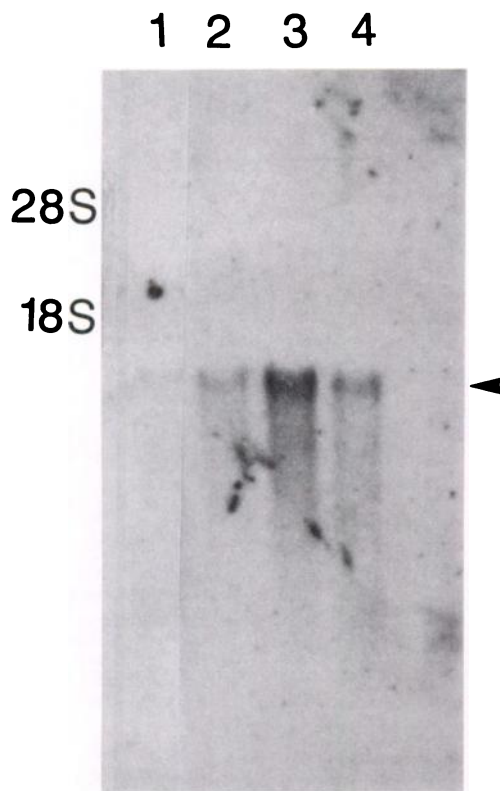


Fig. 3. Northern blot analysis of tumor RNA. An aliquot containing 10 μ g of total RNA was electrophoresed in a 1.4% formaldehyde agarose gel, transferred to a nitrocellulose filter, and hybridized with the human cathepsin L probe labeled with 32 P by random primer extension. Lane 1, KB 3-1; Lane 2, adrenocortical carcinoma; Lane 3, epidermoid lung cancer; Lane 4, large cell anaplastic cancer of the lung.

cathepsin L mRNA of tumors on various blots. The intensity of the bands was comparable to the intensity of the signal for these tumors on slot blots.

DISCUSSION

Several years ago it was demonstrated that malignant transformation of murine cells in tissue culture leads to the overexpression and secretion of a protein which was then named MEP (2, 3). Consequently, MEP was shown to be procathepsin L (25, 26). However, very little information has been available concerning the expression of cathepsin L in human cancers. For this study, we screened a variety of human cancers to determine if overexpression of the cathepsin L gene also occurs in human malignancy. Our results show relatively high expression, compared with normal tissues, of cathepsin L mRNA in many human cancers.

A quantitative slot blot procedure was used to measure cathepsin L RNA levels in various tumors. The validity of this assay has been confirmed in this work by showing that dilutions of the same RNA sample demonstrate proportional decreases in signal intensity after hybridization with a specific radiolabeled cathepsin L cDNA probe (Fig. 1). β -Actin mRNA was used to normalize for the equal loading and quality of the RNA from different tumors. With this assay we could detect a cathepsin L mRNA signal with as low as 0.3 μ g of total RNA from several tumors, indicating it to be a fairly sensitive assay.

The results of the present study demonstrate severalfold higher levels of average cathepsin L mRNA in kidney, testis, colon, and lung tumors (Fig. 1) when compared with normal

tissues and increased levels of expression in cancer of the breast, ovary, adrenal, bladder, and prostate. Similar increased expression of cathepsin L mRNA has been observed by Qian *et al.* (14) in murine tumors of kidney and lung. Increased cysteine protease activity has also been previously reported in gastric tumors, colorectal carcinoma, and breast cancer compared with normal tissues (16, 27–30). Yamaguchi *et al.* (17) detected significantly higher amounts of cathepsin L in a human pancreatic cancer cell line. Our results show a striking increase in the cathepsin L mRNA levels in a pancreatic adenocarcinoma, whereas some pancreatic endocrine tumors showed no noticeable difference in the cathepsin L RNA (Fig. 2). Cysteine protease activities in gastric adenocarcinomas have been reported to vary with the stage of tumor progression (17), so it is possible that the pancreatic endocrine tumors that showed comparatively low levels of cathepsin L mRNA were either benign, which is usually the case, or at an early stage of malignancy.

Proteases have been thought to play a role in tumor invasion and metastasis due to their destructive effects on extracellular matrix. Cathepsin L is more potent than many other proteases in degrading collagen, elastin, laminin, and other components of the basement membrane. It is secreted by malignant cells, stable to denaturation at neutral pH, and carries mannose 6-phosphate which enables it to be taken up by neighboring cells. In this way, cathepsin L can act as an excellent molecular vehicle for the transport of protease activity. Yagel *et al.* (31) demonstrated the suppression of amnion membrane invasion of murine cancer cells by cathepsin L inhibitors. Thus, it is conceivable that the higher expression of cathepsin L in colon, lung, and kidney tumors observed in the present study may be responsible, at least in part, for the invasion of these malignant cells. Furthermore, strategies to inhibit cysteine protease activity may have a role in prevention of cancer invasion or metastases.

Recently cathepsin D has been proposed as a prognostic marker for breast cancer (32, 33). Results of the present study indicate that cathepsin L also is expressed at higher levels in many cancerous tissues as compared with normal tissues (Table 1; Fig. 2). Kidney, testis, and lung tumors, in particular, have elevated expression of cathepsin L. The results reported here suggest that more detailed studies should be undertaken to determine if cathepsin L can be used as a diagnostic or prognostic marker for human cancer.

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