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Enhanced Expression of Cathepsin L in Metastatic Bone Tumors

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Cathepsin L is a kind of cystein proteases which are known to facilitate the invasion and metastasis of tumor cells by degrading the components of basement membrane and extracellular matrix. This study was undertaken to investigate the expression of cathepsin L by Northern blot analysis with radiolabeled cDNA specific for cathepsin L in six normal tissues, two osteosarcoma cell lines, MG-63 and Saos-2, six primary bone tumors and six metastatic bone tumors. In six normal tissues, the highest level of cathepsin L was expressed in liver with the descending order of liver > lung > thymus > ovary > kidney > esophagus. One of the two osteosarcoma cell lines established from the primary sites expressed a high level of cathepsin L mRNA. Out of six primary bone tumors, three (50 %) expressed cathepsin L mRNA, while all (100 %) of six metastatic bone tumors expressed the mRNA. These results demonstrating the higher frequency of expression of cathepsin L in metastatic bone tumors suggest that cathepsin L may participate in tumor invasion and metastasis.

Key Words : *Cathepsin L, Primary bone tumors, Metastatic bone tumors*

INTRODUCTION

The invasion and metastasis of tumor cells is one of the most frequently developed intrinsic problems that cause mortality in clinical medicine. In recent years, the process of tumor metastasis and invasion was known to be regulated by the complicated interactions between tumor cells and host cells (Liotta, 1986 ; Fidler, 1990 ; Stetler-Stevenson et al., 1993). The process of invasion and metastasis begins with the detachment of tumor cells from the primary site followed by invasion to adjacent structures, such as basement membrane and extracellular matrix (Poste and Fidler, 1980 ; Hart et al., 1989).

In previous studies, the invasive and metastatic potentials of tumor cells are closely associated with the production of various proteases with degradative activity, such as matrix metalloproteinases, collagenases and plasminogen activator (Liotta et al., 1982 ; Mullins and Rohrlich, 1983 ; Duffy, 1992). In addition to the above proteases, a unique group of protease,

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the cathepsin family, was recently identified as another kind of potent protease (Barrett and Kirschke, 1981 ; Sloane and Honn, 1984). Cathepsin, a lysosomal acid cystein protease present in nearly all types of cells, is believed at present to play an important role in the degradation and turnover of intracellular proteins in normal cells and invasion and metastasis in tumor cells. The cathepsin family is composed of several subtypes, among which cathepsin L was reported to be a biologically important and strong protease (Chauhan et al., 1991). In this study, we investigated the expression of cathepsin L in normal tissues, osteosarcoma cell lines and tissues of primary and metastatic bone tumor by Northern blot analysis.

MATERIALS AND METHODS

Tissue specimens

Specimens used for the study of the expression of mRNA for cathepsin L were six normal tissues, six primary bone tumor tissues and six metastatic bone tumor tissues, which were obtained surgically at the Korea Cancer Center Hospital.

Primary bone tumors consisted of three osteosarcoma, one Ewing's sarcoma, one giant cell tumor and one chondrosarcoma. Primary lesions of six metastatic bone tumors were thyroid cancer in two cases, soft tissue sarcomas in two cases, lung cancer in one case and metastatic adenocarcinoma of unknown primary site in one case. The clinical characteristics of these 12 patients are listed in Table 1.

Two osteosarcoma cell lines were maintained in RPMI-1640 medium (Gibco Laboratory, Grand Island, NY, USA) supplemented with 10 % fetal bovine serum (Gibco) at 37℃ in a humidified atmosphere of 5 % CO2. Cells were washed with cold phosphate buffered saline and treated with solution D to extract total RNA.

Isolation of RNA and Northern blot analysis

All tissue specimens and osteosarcoma cells were frozen with liquid nitrogen immediatedly after their surgical removal and stored at −70℃ until the experiment. After the pulverization of frozen tissues and cells, total cellular RNAs were extracted by the guanidine thiocyanate-phenol-chloroform extraction method. Twenty μg of RNA extracted were electrophoretically size-fractionated on 1 % agarose gel containing formaldehyde and transferred onto nylon membranes (Schleicher & Schuell, Germany) by capillary action in 10x standard saline citrate(SSC) overnight. Before blotting, each gel was stained by ethidium bromide to visulalize ribosomal RNA by ultraviolet (UV)-lighting. The membranes were washed in 2x SSC and UV-cross-linked using UV Stratealinker 2400 (Stratagene, USA). The membranes were prehybridized overnight at 42℃ in 50 % formamide, 1x Denhardt's solution, 20mM sodium phosphate, 0.1 % sodium dodecyl sulfate (SDS), 100 μg/ml of salmon sperm DNA, and 6× SSC.

The cDNA probes used for Cathepsin L was kindly provided by Gottesmann MM, National Cancer Insti-

Table 1. Characteristics of patients with bone tumor and expresseion of cathepsin L mRNA

Case No.	Sex	Age	Primary Site	Histology	Cathepsin L expression
Primary bone tumor					
1	M	47	bone	chondromyxoid fibroma	+
2	M	14	bone	osteosarcoma	−
3	M	39	bone	osteosarcoma	+
4	M	19	bone	osteosarcoma	++
5	M	49	bone	Giant cell tumor	−
6	F	17	bone	Ewing's sarcoma	−
Metastatic bone tumor					
1	F	36	thyroid	carcinoma	+
2	F	58	thyroid	adenocarcinoma	+
3	M	22	lung	carcinoma	++
4	F	43	soft tissue	sarcoma	+
5	F	33	soft tissue	sarcoma	+
6	F	51	unknown	adenocarcinoma	+

tute, NIH, USA. The mambrane was hybridized at 42℃ with the probe labeled with [^{32}P]-dCTP by the nick-translation method(BM, Germany). Hybridized filters were washed in 2x SSC, 0.1 % SDS at room temperature for 30 min. and 0.1x SSC, 0.1 % SDS for 30 min. at 65℃ before autoradiography at −70℃ with intensifying screens. The expression of mRNA was graded as −, + and ++ according to the intensity of the bands as compred with the band of liver tissue. The cases with + or ++ were considered to be enhanced expression.

RESULTS

The mRNA levels of cathepsin L were determined by northern blot analysis. To evaluate the message levels for RNA, ribosomal 18S and 28S RNA were stained by ethidium bromide. In normal tissues tested, the highest level of cathepsin L was found in liver, with the order of liver > lung > thymus > ovary > kidney > esophagus (Fig. 1-A). The results of northern blot analyses in two osteosarcoma cell lines, MG-63 and Saos-2, are shown in Fig. 1-B, demonstrating that a high level of cathepsin L was detected in Saos-2.

The expression of cathepsin L in six samples of primary and six samples of metastatic bone tumors is shown in Fig. 2. As shown in Table 2, cathepsin L was expressed in three (50 %) out of six primary

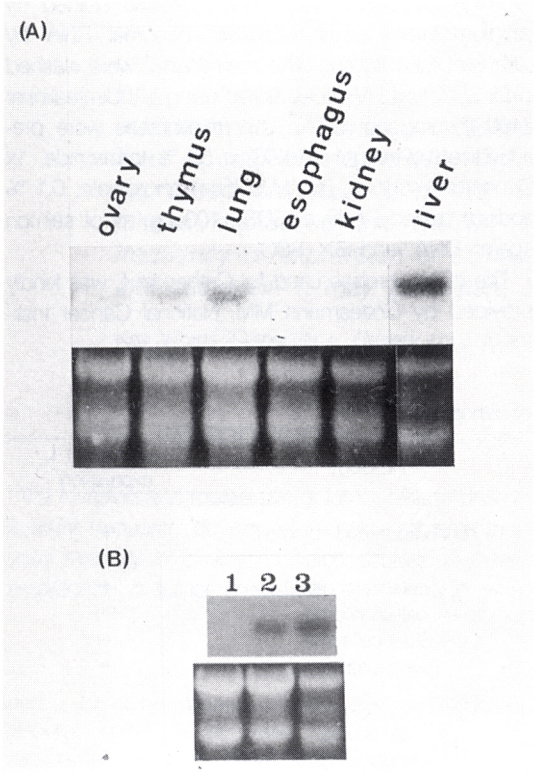

Fig. 1. Cathepsin L mRNA expression by Northern blot anaysis in six normal tissues(A) and two osteosarcoma cell lines(B), Lane 1 : MG-63, Lane 2 : Saos-2, Lane 3 : normal liver(control).

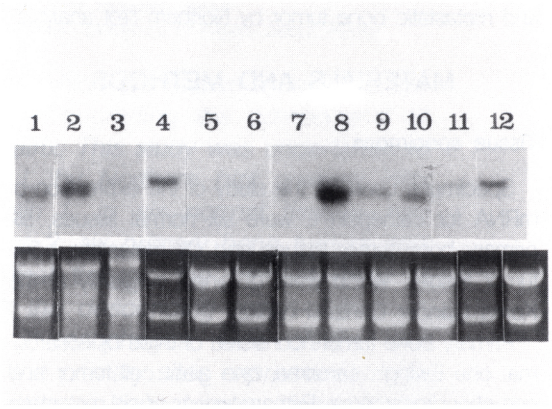

Fig. 2. Cathepsin L mRNA expression by Northern blot analysis in six primary and six metastatic bone tumor tissues, Lane 1 : chondromyxoid fibroma, Lane 2,3,4 : osteosarcoma, Lane 5 : giant cell tumor, Lane 6 : Ewing's sarcoma, Lane 7,9 : metastatic bone tumor from thyroid, Lane 8 : metastatic tumor from lung, Lane 10 : metastatic adenocarcinoma of unknown primary site, Lane 11,12 : metastatic tumor from soft tissue sarcoma.

Table 2. Expression of mRNA for cathepsin L in six primary and six metastatic bone tumors

Tumors	Expression of cathepsin L mRNA		
	−	+	++
primary bone tumors	3(50%)*	2(33%)	1(17%)
chonromixoid fibroma	0	1	0
osteosarcoma	1	1	1
Giant cell tumor	1	0	0
Ewing's sarcoma	1	0	0
metastatic bone tumors	0(0%)	5(83%)	1(17%)
from thyroid	0	2	0
from lung	0	0	1
from soft tissue sarcoma	0	2	0
from unknown adenocarcinoma	0	1	0

* : number of patients tested(%).

bone tumor, while expressed in all (100 %) out of six metastatic bone tumors. Based on the higher expression of cathepsin L mRNA in metastatic bone tumors compared with primary bone tumors ($p < 0.05$ by Chi-Square test), we speculate that cathepsin L may involved in tumor invasion and metastasis.

DISCUSSION

Cathepsin is a group of cysteine proteases which have the property of degradation of various intracellular and extracellular proteins in physiologic state (Barrett and Kirschke, 1981 ; Sloane and Honn, 1984). Cathepsin is composed of several classes, such as cathepsin A, B, C, D, H, L and N. Of the cathepsin subtypes , cathepsin L was reported to be more potent than the other cathepsin classes in degrading extracellular matrix components, such as collagens, fibronecin and proteoglycans, and basement membrane components, such as collagens and laminin (Chauhan et al., 1991). Cathepsin L was also reported to have proteolytic activity aganist a variety of proteins including serum proteins, cytoplasmic proteins, nuclear proteins and bone matrix (Goto et al., 1993 ; Kakegawa et al., 1993 ; Ohsawa et al., 1993). Although the amount of cathepsin L is variable, nearly all types of cells contain cathepsin L which is thought to play a primary role in protein turnover.

In different types of tumor tissues, cancers in general express higher levels of cathepsin L mRNA than do normal tissues, are reported (Chauhan et al., 1991). Moreover, Yagel et al. demonstrated the suppression of amnion membrane invasion of murine cancer cells by cathepsin L inhibitors. Thus, the elevated levels of intracellular cathepsin L are thought to be closely related, at least in part, to the biochemical processes underlying tumor invasion and metastasis. On the other hand, cathepsin L is also reported to be associated with malignant transformation (Gottesmann, 1978 ; Chung, 1990 ; Kane and Gottesman, 1990). The mechanisms by which cathepsin L mediates tumor invasion, metastasis and malignant transformation are poorly understood, although a slight change in the sugar structure of cathepsin L produced by tumor cells is suggested to be the mechanism (Chung, 1990).

In this study, six normal tissues were examined to investigate the expression of cathepsin L, demonstrating the variable degree of mRNA expression. Concerning the mechanisms of the difference, we cannot explain the exact mechanism of the different levels of cathepsin L between the tissues at present. Our results for the expression of cathepsin L in normal tissues are in agreement with previous reports (Chauhan and Goldstein, 1991). They showed that liver tissue expressed the highest level of cathepsin L, while esophagus had the lowest level.

Goto et al.(1993) exmined the localization of cathepsins B, D and L in the osteoclasts of rat alveolar and femoral bones by using immunohistochemical staining method. Cathepsins B and L were detected along the bone resorption lacunae. And this report suggested that cathepsins B and L directly and effectively participate in the degradation of the bone matrix. Because of the known potent proteolytic activity of cathepsin L, it seems reasonable that cathepsin L is closely related to the degradation of organic constituents of the bone matrix. Recently, cathepsin L was reported to regulate bone resorption, therefore cathepsin L is the main proteinase responsible for bone collagen degradation (Kakegawa et al, 1993). However, little data has been available so far, to our knowledge, on the cathepsin L expression in bone tumors.

Therefore, in this study, to identify cathepsin L play a role in malignancy of bone tumor, we examined the expression of cathepsin L in two osteosarcoma cell lines, six primary and six metastatic bone tumors. The two osteosarcoma cell lines were established from the primary sites, among which one cell line showed a high level of the cathepsin L expression. On the other hand, cathepsin L was expressed in all metastatic bone tumors, but expressed in 50 % of primary bone tumors. From the results demonstrating that the high frequency of expression of cathepsin L in metastatic bone tumors compared to primary bone tumors, we guess that cathepsin L may have relation with the tumor invasion and metastasis of bone tumors. In order to draw a conclusion on this point, however, more detailed study should be undertaken, including comparison of cathepsin L activity between primary and metastatic tumor against a large number of samples and gene transfection study, to determine if cathepsin L play a key role in bone tumor invasion and metastasis.

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