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사람 위선암에서 cathepsin L의 발현증가

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=Abstract=

Increased Expression of Cathepsin L in Human Gastric Adenocarcinoma

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Cathepsin L, a lysosomal cysteine protease, is known to play an important role in cancer invasion and metastasis by degrading the components of basement membrane and extracellular matrix. The mRNA expression of cathepsin L was determined by Northern blot analysis using a radiolabeled cDNA specific for cathepsin L in five human gastric adenocarcinoma cell lines and five surgical specimens of primary gastric adenocarcinomas, their metastatic lymph nodes and matched adjacent normal mucosae. The mRNA of cathepsin L was expressed in all of the four cell lines established from the metastatic sites, SNU-5, SNU-16, MKN-45 and Kato III, while not detected in one cell line established from the primary site, AGS. The mRNA was expressed in all of the five primary and five metastatic cancer specimens tested, while it was not detected in all matched normal mucosae. The intensities of the mRNA expressions, however, did not show the consistent pattern between primary sites and metastatic lymph nodes. These results suggest that cathepsin L may have the other function in addition to facilitation of the invasion and metastasis during the development and progression of stomach cancer.

Key words : Cathepsin L, Gastric adenocarcinoma, Metastasis, Lymph node

INTRODUCTION

In recent years, various attempts have been made to ellucidate the mechanisms of invasion and metastasis of cancer cells, because they are critical factors of morbidity and mortality in most of

human solid cancers including gastric adenocarcinoma[1,2]. The process of invasion and metastasis of cancer cells has been reported to be closely related to the increased activity of various preoteases, such as matrix metalloproteinases, plasminogen activator and collagenases[3-6]

Recently, cathepsin has been indentified as one of the proteases belonging to this classification[7,8]. Cathepsin is a family of lysosomal acid cystein proteases present in nearly all types of cells. Among the cathepsin family, cathepsin L was reported to have the most strong protease activity[9,10]. Because of the potent protease activity, cathepsin L is now generally accepted to play an important role in cancer invasion and metastasis by degrading the components of basement membrane and extracellular matrix[11].

Gastric adenocarcinoma is the most common cause of cancer death in Korea. However, the mechanisms of invasion and metastasis of gastric cancer are still unclear. As one of the studies to ellucidate the mechanisms, we examined the mRNA expression of cathepsin L, using Northen blot analysis, in surgical specimens obtained from five patients with gastric adenocarcinoma as well as five human gastric adenocarcinoma cell lines.

MATERIALS AND METHODS

Cells and tissue specimens

Gastric cancer cell lines employed in these experiments were SNU-5, SNU-16, MKN-45, Kato III and AGS. SNU-5 and SNU-16 were kindly provided by professor J-G Park, Seoul National University, Seoul, Korea. MKN-45 and Kato III were gifted by Dr. N. Saijo, Japan National Cancer Center Hospital, Tokyo, Japan. AGS was obtained from American Type Culture Collection. Characteristics of these cell lines are listed in Table 1. All cell lines were propagated in RPMI-1640 medium (Gibco Laboratory, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco), 100 U/ml of penicillin and 100 μg/ml of streptomycin at 37℃ in a humidified atmosphere of 5% CO_2. Five specimens of primary gastric adenocarcinoma, their metastatic lymph nodes and matched adjacent normal mucosae were obtained during operation. All gastric cell lines and tissue specimens obtained were washed with phosphate buffered saline and stored at −70℃ until experiment.

Table 1. Characteristics of human gastric cancer cell lines

	Histology	Sex	Source	Prior treatment
SNU-5	Ad	F	ascites	+
SNU-16	Ad	F	ascites	−
MKN-45	Ad	F	liver	+
Kato III	Ad	M	pleural effusion	+
AGS	Ad	F	stomach	−

Ad : adenocarcinoma

Northern blotting

After the pulverization of frozen cells and tissues, total cellular RNAs were extraced by the guanidine thiocyanate-phenol-chloroform extraction method. Twenty μg of RNA extracted were electrophoresed on 1% agarose-formaldehyde gel and blotted onto nylon filter membranes (Schleicher & Schuell, Dassel, Germany) by capillary transfer in 10xstandard 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 2xSSC and UV-cross-linked using UV Stratealinker 2400 (Stratagene, Menasha, WI, USA). The membranes were prehybridized overnight at 42℃ in 50% formamide, 5x Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), 100 μg/ml of salmon sperm DNA, and 6xSSC.

The radiolabeled 1.8 kilobase cDNA probe specific for cathepsin L was kindly provided by Go-

ttesmann MM, National Cancer Institute, NIH, USA. The membrane was hybridized at 42℃ with the probe labeled with [^{32}P]−dCTP by the nick−translation method. Hybridized filters were washed in 2xSSC, 0.1% SDS at room temperature for 30 min and 0.1xSSC, 0.1% SDS at 65℃ before autoradiography at −70℃ with intensifying screens. To evaluate the message levels for mRNA, ribosomal 18S and 28S RNA were stained by ethidium bromide. The expression of the mRNA was graded as −, + and ++ by intensity of the bands.

RESULTS

The mRNA levels of cathepsin L were determined by Northern blot analysis in four human gastric cancer cell lines established from metastatic sites, SNU−5, SNU−16, MKN−45 and Kato III, and one gastric cancer cell line established from primary site, AGS. All of the four cell lines established from metastatic sites expressed mRNA of cathepsin L; high level (++) in SNU−5 and low level (+) in SNU−16, MKN−45 and Kato III (Fig. 1). In AGS, the mRNA expression was not detected.

The expression of the mRNA was observed in all of the five primary and five metastatic cancer specimens, while not detected in all matched five normal mucosae (Table 2)(Fig. 2). As shown in Fig. 2, however, the intensities of the mRNA expressions did not show the consistency between primary sites and metastatic lymph nodes. The mRNA level of the metastatic lymph node was higher than that of the primary site in one patient, and lower in two patients, while it was similar in two patients.

Table 2. Expression of mRNA for cathepsin L in five human gastric adenocarcinoma cell lines and five specimens of primary gastric adenocarcinomas, their metastatic lymph nodes and matched normal mucosae

Specimen	Expression of cathepsin L mRNA		
	−	+	++
Gastric cancer cell lines(n=5)	1(20%)*	3(60%)	1(20%)
Surgically resected specimens(n=5)			
primary sites	0(0%)	2(40%)	3(60%)
metastatic lymph nodes	0(0%)	3(60%)	2(40%)
normal mucosae	5(100%)	0(0%)	0(0%)

* number of patients (%)

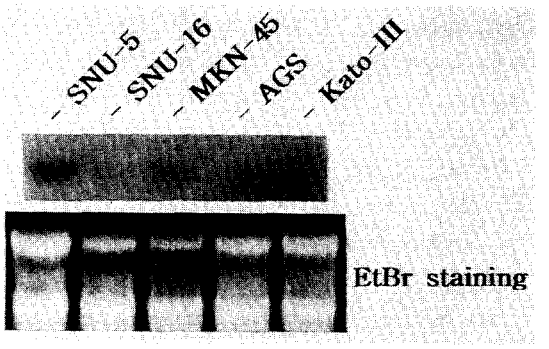

Fig. 1. Cathepsin L mRNA expression by Northern blot analysis in five gastric adenocarcinoma cell lines, SNU−5, SNU−16, MKN−45, AGS and Kato III.

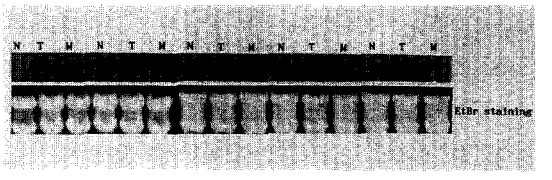

Fig. 2. Cathepsin L mRNA expression by Northern blot analysis in five specimens of primary gastric adenocarcinomas, their metastatic lymph nodes and matched normal mucosae.

DISCUSSION

Cathepsin is a lysosomal acid cystein protease which is responsible for various intracellular protein turnover of normal cells and the invasion and metastasis of cancer cells. Several subtypes of cathepsin have been identified to have the proteolytic activity so far, such as cathepsin A, B, C, D, H, L and N[7,12]. Among the subtypes, cathepsin L

was reported to be more potent than the other cathepsin subtypes in degrading collagen, elastin, laminin, and other components of the basement membrane[9,10].

Although cathepsin L is synthesized and secreted by both normal cells and cancer cells, cancer cells usually produce larger amount of cathepsin L than normal cells[10]. In addition, cathepsin L secreted by cancer cells is fairy stable to denaturation and carries mannose 6-phosphate[9]. Through the process of uptake of mannose 6-phosphate by neighboring cells, its protease activity can be transported to the surrounding tissues. On the other hand, amnion membrane invasion by murine cancer cells was reported to be suppressed by cathepsin L inhibitors[13]. On the basis of these results, cathepsin L is now believed to play an important role in invasion and metastasis of cancer cells.

On the other hand, cathepsin L was also reported to be associated with malignant transformation [14,15]. The sugar structure of cathepsin L produced by cancer cells was known to be slightly different from that produced by normal cells. The different sugar structure was proposed to be related to malignant transformation[16].

In addition to cancer cells, normal cells may produce various amounts of cathepsin L. To determine the production of cathepsin L in normal cells, we investigated the mRNA of cathepsin L in six normal tissues and demonstrated that the highest level of the mRNA was observed in liver, with the order of liver > lung > thymus > ovary > kidney > esophagus[11]. In this study, we could not detect the mRNA of cathepsin L in all five specimens of normal gastric mucosae. In an agreement with this result, Chung[16] reported that cathepsin L activity was much lower in normal gatric mucosal tissue than gastric cancer tissue.

In this study, the expression of cathepsin L mRNA was examined in both gastric cancer cell lines and cancer tissues surgically resected. All gastric cancer cell lines established from metastatic sites expressed the mRNA, while one cell line established from primary site did not express it. On the other hand, the mRNA was expressed in all tissues of primary sites and metastatic lymph nodes, suggesting that cathepsin L might be produced during transformation or at an early stage of progression, not during acquiring the metastatic potential. Moreover, the intensity of the mRNA did not show a tendency to increase in metastatic sites compared with primary sites.

Although the number of cell lines tested was too small to draw a conclusion on the role of cathepsin L, these results may suggest that cathepsin L may not be associated with cancer invasion and metastasis or have the other function in the development of stomach cancer, because it is produced in all cancer tissues isolated from the primary sites. It is possible that cathepsin L may be produced by different mechanisms in stomach cancer from the other types of cancer and may play a different role with the type of cancers. Therefore, further studies will be necessary to come to a conclusion on the role of cathepsin L in stomach cancer.

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=국문 초록=

사람 위선암에서 cathepsin L의 발현증가

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cathepsin L은 lysosomal cysteine 단백분해효소로서 기저막(basement membrane)과 세포외기질(extracellular matrix)을 파괴하여 암세포의 침윤과 전이에 중요한 역할을 하는 물질로 알려져 있다. 이러한 cathepsin L에 대한 mRNA 발현도를 5개의 사람 위선암(gastric adenocarcinoma) 세포주와 5명의 위선암 환자에서 채취한 조직에서 방사능으로 표지된 cathepsin L 특이 cDNA를 사용한 Northern blot법으로 측정하였다. 위암의 전이병소에서 수립한 세포주인 SNU-5, SNU-16, MKN-45와 Kato III에서는 cathepsin L mRNA가 발현되었으나 원발병소에서 수립한 AGS 세포주에서는 mRNA의 발현이 관찰되지 않았다. 5명의 위암 환자에서는 원발병소, 전이가 확인된 임파절 및 암 근처 정상 위점막에서 각각 조직을 채취하여 cathepsin L mRNA의 발현을 측정하였다. 원발병소와 전이병소에서는 모두 cathepsin L mRNA가 발현되었으나 정상 위점막조직에서는 전예에서 mRNA 발현이 관찰되지 않았다. 한편 mRNA의 발현도는 1예에서는 전이병소가 원발병소에 비해 높았으나, 2예에서는 전이병소에서 발현도가 낮았으며, 나머지 2예에서는 원발병소와 전이병소 사이에 차이가 없어, 원발병소와 전이병소 사이에 mRNA의 발현도의 일관성 있는 경향은 관찰되지 않았다. 이상의 결과는 cathepsin L은 위암의 발생과 진행에 있어 암세포의 침윤과 전이를 촉진하는 것 이외에 또 다른 역할을 할 가능성을 시사하고 있다고 사료된다.

Key words : cathepsin L, 위선암, 전이, 임파절