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Immunohistochemical Analysis of Cathepsin B Expression in Human Lung Adenocarcinoma: The Role in Cancer Progression

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Production of cathepsin B by tumor cells has been linked to metastatic potential in several experimental models. Sections of 95 primary lung adenocarcinomas were examined for expression of cathepsin B using a standard avidin-biotin immunohistochemical technique. Staining for cathepsin B was observed in 22.1% of all cases and 28.0% of those of the Clara cell type. In Clara cell adenocarcinomas, cathepsin B expression correlated with positive lymph node status, presence of distant metastases, and poor prognosis ($P<0.05$). However, no correlation with clinical outcome was observed in other cell types. Our data suggest that cathepsin B may be involved in invasion and metastasis in Clara cell lung adenocarcinoma.

Key words: Cathepsin B — Immunohistochemistry — Lung adenocarcinoma

Cathepsin B (EC 3.4.22.1) is a cysteine proteinase found mainly within the lysosomes of normal cells, where it is thought to play a role in cellular and extracellular protein turnover.[1] Cathepsin B is capable of degrading extracellular matrix components, such as proteoglycans, collagens, and fibronectin, as well as basement-membrane-specific components including type IV collagen and laminin.[2] High levels of cathepsin B are present in many human tumors, and are thought to contribute to biochemical processes underlying tumor metastasis.[3,4]

In this study, we examined the expression of cathepsin B in lung adenocarcinomas by immunohistochemical staining to determine its role in tumor malignancy.

MATERIALS AND METHODS

Patients Tumor specimens were obtained from primary lung adenocarcinomas of 95 patients treated surgically between 1978 and 1991 at the National Defense Medical College Hospital. Forty-nine patients were male and 46 were female. The age range was 36 to 85 years (mean, 63.0 years). TNM staging was defined according to the criteria of the Japan Lung Cancer Society.[5]

Well and moderately differentiated adenocarcinomas were classified cytologically into 5 subtypes according to Shimosato's criteria[6]: bronchial surface epithelial cell type, bronchial gland cell type, goblet cell type, Clara cell type, and type II alveolar epithelial cell type (Table I).

Immunohistochemistry Formalin-fixed, paraffin-embedded sections were stained with hematoxylin and eosin. All materials were reviewed retrospectively by one pathologist (S.A.) and histologic and cytologic diagnoses were made.

Immunohistochemical staining was performed on paraffin sections using an avidin-biotinyl peroxidase complex method. Briefly, deparaffinized, rehydrated sections were treated with 0.6% hydrogen peroxide in methanol for 30 min to blook endogenous peroxidase activity. The slides were preincubated with 2% normal rabbit serum for 1 h. Excess serum was drained and sections were incubated with sheep anti-human cathepsin B antibody (Binding Site Ltd., England) at a dilution of 1:250. The sections were incubated with biotinylated rabbit anti-sheep secondary antibody (Vector Laboratories Inc., Burlingame, CA) diluted 1:250 for 30 min, followed by incubation with horseradish peroxidase-conjugated streptavidin (Vector) at 1:250 in phosphate-buffered saline (PBS) for 30 min. The peroxidase reaction was performed using 0.02% 3,3′-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 *M* Tris-HCl buffer, pH 7.4. Finally, nuclear counterstaining was performed with 1% methyl green. Between each step, the slides were washed two times (10 min each) in PBS, and all steps in the protocol were performed at room temperature. PBS was used as the diluent of antibodies and peroxidase. Macrophages in the section served as positive controls. For negative controls, each section was stained without using primary antibody.

Immunoreactivity was graded as − to ++ according to the percentage of positive cells and the intensity of staining. The cases in which more than 50% of cells were stained intensely (dark brown or ++) or moderately (brown or +) were considered positive; and those stained weakly (light brown or ±) or not stained were considered negative (−). Only strong and moderate reactions were judged to be true positive because weak reactions might include false-positive results. According to this grading protocol, two independent pathologists

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(S.A. and S.T.) examined all the immunostained specimens randomly to make the grading as objective as possible.

Statistical analysis Statistical significance was evaluated by means of the chi-square test, with $P<0.05$ as the criterion of statistical significance. Curves for overall survival were drawn according to the Kaplan-Meier method,[7] and differences between curves were analyzed by the generalized Wilcoxon test.[8]

RESULTS

In normal lung tissue, macrophages stained intensely. Of the 95 primary lung adenocarcinomas, 21 (22.1%) were positive for cathepsin B. Positive cells showed diffuse cytoplasmic staining (Fig. 1).

Table I. Materials

	No. of cases (%)[a]
Well differentiated adenocarcinoma[b]	79
Bronchial surface epithelial type	9 (11.4)
Bronchial gland cell type	14 (17.7)
Goblet cell type	5 (6.3)
Clara cell type	50 (63.3)
Type II alveolar epithelial cell type	1 (1.3)
Poorly differentiated adenocarcinoma	16
Total	95

a) The number and percentage of cases of well-differentiated adenocarcinoma.
b) Including moderately differentiated.

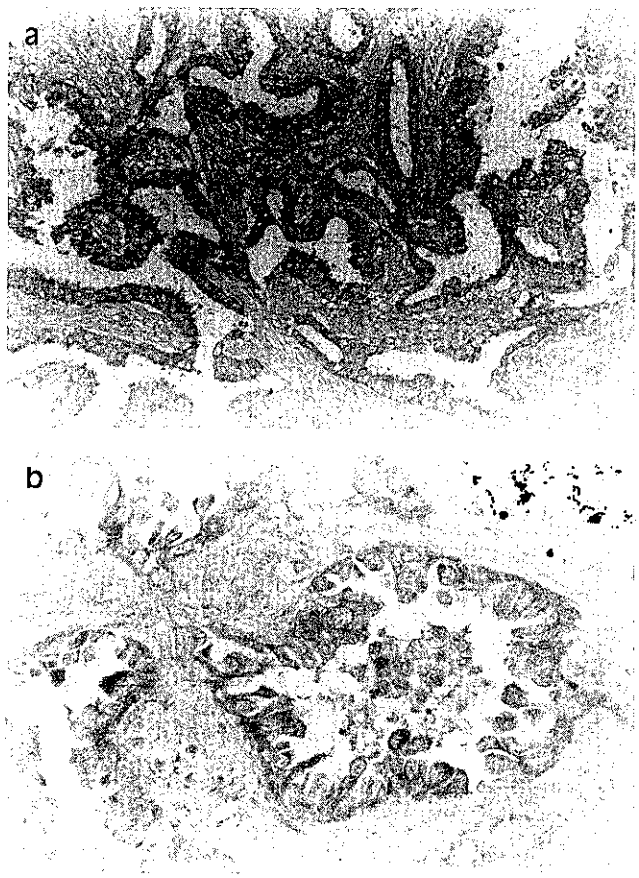

Fig. 1. Immunohistochemical staining for cathepsin B in lung adenocarcinoma. Positive cells showed diffuse cytoplasmic staining. a: ×100, b: ×400.

Table II. Correlation between Cathepsin B Expression and Pathologic Stage in Lung Adenocarcinoma

Cell type	No. of cases (%)[a]			*P* value
Stage	I, II	III, IV	Total	
Surface[b]	1/2 (50.0)	1/7 (14.3)	2/9 (22.2)	NS[h]
Gland[c]	0/3 (0)	2/11 (18.2)	2/14 (14.3)	NS
Goblet[d]	1/3 (33.3)	0/2 (0)	1/5 (20.0)	NS
Clara[e]	2/21 (9.5)	12/29 (41.4)	14/50 (28.0)	<0.05[i]
Type II[f]	0/1 (0)	0/0 (0)	0/1 (0)	NS
Poorly[g]	1/5 (20.0)	1/11 (9.1)	2/16 (12.5)	NS
Total	5/35 (14.3)	16/60 (26.7)	21/95 (22.1)	NS

a) The numerator is the number of cathepsin B-positive cases of a particular cytologic type; the denominator is the number of cases of a particular pathologic stage.
b) Bronchial surface epithelial type.
c) Bronchial gland cell type.
d) Goblet cell type.
e) Clara cell type.
f) Type II alveolar epithelial cell type.
g) Poorly differentiated adenocarcinoma.
h) NS, not significant.
i) Significantly different between stages I, II and stages III, IV.

The percentage of cathepsin B-positive cells varied according to cytologic type (Table II). The Clara cell type showed the highest positive rate (28.0%), and only in this subtype was cathepsin B staining significantly correlated with pathologic stage ($P<0.05$). The incidence of regional lymph node metastasis in Clara cell type was significantly higher in cathepsin B-positive cases (76.9%) than in negative cases (41.7%) ($P<0.05$). The incidence of distant metastasis was also significantly higher in cathepsin B-positive cases (35.7%) than in negative cases (8.3%) ($P<0.05$) (Table III).

Table III. Correlation between Cathepsin B Expression and Regional Lymph Node and Distant Metastatic Status in Lung Adenocarcinoma of the Clara Cell Type

	No. of cases (%)[a]		*P* value
	Cathepsin B immunoreactivity		
	+	−	
Lymph node status			<0.05
positive	10/13 (76.9)	15/36 (41.7)	
negative	3/13 (23.1)	21/36 (58.3)	
Distant metastasis			<0.05
metastasis	5/14 (35.7)	3/36 (8.3)	
no metastasis	9/14 (64.3)	33/36 (91.7)	

a) The numerator is the number of cases with or without metastasis in lymph node or distant organ; the denominator is the number of cathepsin B-positive or negative cases.

In the Clara cell type tumors, there was a significant difference in the overall survival curves between the cathepsin B-positive and negative groups ($P<0.05$) (Fig. 2). In all other cell types, no correlation was found between cathepsin B staining and clinicopathologic features.

DISCUSSION

Tumor cell invasion and metastasis are multistep events which require the integration of several factors. For a tumor to be invasive, cells must penetrate the basement membrane and move into surrounding normal tissues.[9-11] In order for cancer cells to degrade the extracellular matrix, including the basement membrane, the release of certain proteolytic enzymes appears to be necessary. Indeed, tumors are known to contain and secrete a variety of specific proteinases, including plasminogen activators,[12-14] lysosomal hydrolases, and matrix metalloproteases.[15]

Cathepsin B, a lysosomal cysteine endopeptidase, can degrade extracellular matrix components, such as proteoglycans, collagens, and fibronectin, as well as basement membrane-specific components including type IV collagen and laminin,[2] and has been implicated in tumorigenesis, tumor invasion, and metastasis.[16-18] In addition to direct degradation of the extracellular matrix, cathepsin B may be involved in the activation of collagenase.[13]

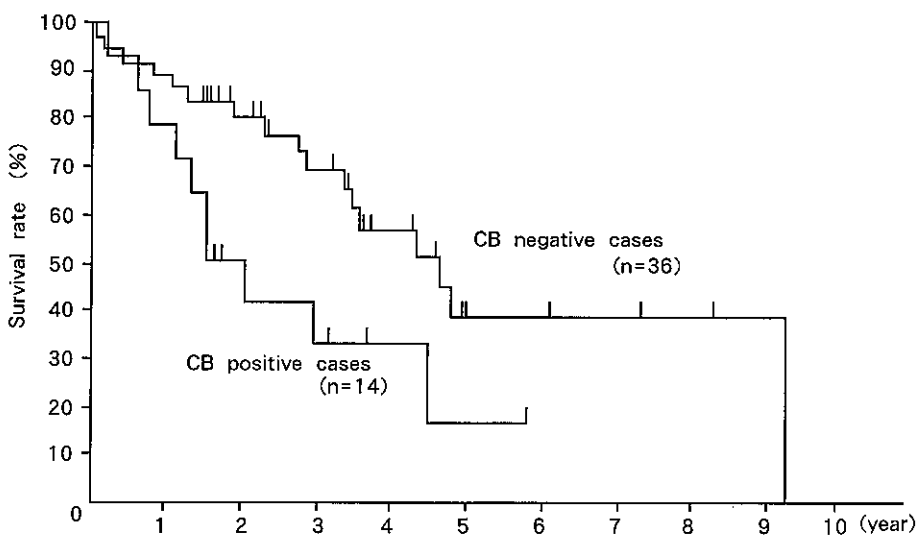

Fig. 2. Kaplan-Meier survival curves of patients with lung adenocarcinoma of the Clara cell type with regard to cathepsin B expression. Cathepsin B-positive cases had a poorer survival rate than negative cases ($P<0.05$). Tic marks indicate surviving patients, CB: cathepsin B.

In malignant cells, cathepsin B is associated with plasma membranes.[2, 4, 19] The localization of cathepsin B was unclear in our study, but since its staining pattern in lung cancer cells was diffusely cytoplasmic rather than granular, it was most likely membrane-associated, as described previously.[19]

We found that cathepsin B staining was significantly correlated with pathologic stage only in Clara cell type, but not in other cell types. Lung adenocarcinomas are, however, heterogeneous as to histologic type, cytologic type, and behavior. A possible explanation for the discrepancy in correlating cathepsin B expression with clinical outcome could be differences in the heterogeneous phenotypes of lung adenocarcinoma.

We have shown that immunohistochemical staining of cathepsin B correlated significantly with pathologic stage, regional lymph node status, presence of distant metastases, and overall survival in primary lung adenocarcinoma of the Clara cell type. These results suggest that cathepsin B may play an important role in the progression and dissemination of carcinoma of this cell type.

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