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Serum Level of Cathepsin B and its Density in Men with Prostate Cancer as Novel Markers of Disease Progression

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Abstract. *Background: Cathepsin B has been shown to play an important role in invasion and metastasis of prostate cancer. The objective of this study was to determine whether serum levels of cathepsin B and its density (cathepsin B-D) could be used as predictors of disease extension as well as prognosis in patients with prostate cancer. Materials and Methods: Serum levels of cathepsin B in 60 healthy controls, 80 patients with benign prostatic hypertrophy (BPH) and 120 patients with prostate cancer were measured by a sandwich enzyme immunoassay. Cathepsin B-D was calculated by dividing the serum levels of cathepsin B by the prostate volume, which was measured using transrectal ultrasonography. We subsequently analyzed the association between these two factors and several clinicopathological factors. Results: The mean values of cathepsin B and cathepsin B-D in patients with prostate cancer were significantly higher than those in healthy controls and BPH patients. Moreover, the cathepsin B and cathepsin B-D levels in patients with metastasis were significantly elevated compared with those in patients without metastasis. Among patients undergoing radical prostatectomy, the levels of cathepsin B and cathepsin B-D in those with pathologically confirmed extraprostatic disease were significantly higher than in patients with organ-confined disease. However, there was no significant association between the elevation of cathepsin B and cathepsin B-D levels and cause-specific survival in prostate cancer patients. Conclusion: These findings indicate that the elevation of serum cathepsin B and cathepsin B-D could be used as novel predictors of disease extension, but not survival, in patients with prostate cancer.*

Prostate cancer is the most frequently diagnosed malignancy in men in Western industrialized countries and is the second leading cause of cancer-related death (1). Recent progression in the fields of diagnosis and follow-up using prostate-specific antigen (PSA) and its associated parameters have contributed to early detection and accurate prediction of prognosis (2, 3). However, despite these advances, more than 50% of patients still show evidence of advanced disease at the time of diagnosis, and the currently available parameters are not correlated with the clinical course in some patients during progression to hormone-refractory disease (4); hence, there is a pressing need to develop a novel diagnostic and monitoring marker system in order to further improve the prognosis of patients with prostate cancer.

Cancer cells generally possess a high degree of proteolytic activity, resulting in the enhanced ability to degrade and subsequently invade surrounding normal tissues (5). Several proteolytic enzymes have been shown to be involved in the degradation of the extracellular matrix and basement membrane (6-8). Among them, cathepsin B, a lysosomal cystine protease, is one of the most important enzymes for invasion and metastasis with increased expression in various kinds of malignant tumors (9-12). Furthermore, measurement of serum levels of cathepsin B could serve as a useful prognostic marker for patients with certain types of malignant tumor (13, 14). Consistent with clinical investigations, several experimental studies also demonstrated crucial roles of cathepsin B in cancer progression (15-17). These findings suggest that cathepsin B could be a potential diagnostic marker as well as a useful indicator of cancer progression.

Recently, several studies have reported the close association between prostate cancer extension and cathepsin B expression (18-22); however, to our knowledge, no report has analyzed serum levels of cathepsin B in men with prostate cancer. Accordingly, in the present study, we measured the serum levels of cathepsin B and its density (cathepsin B-D) in prostate cancer patients, and investigated the relationship between these levels and several clinicopathological factors.

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Table I. *Serum cathepsin B and cathepsin B-D levels in healthy controls and patients with BPH and prostate cancer.*

	Number of subjects	Cathepsin B (ng/ml)		Cathepsin B-D (ng/ml^2)	
		Mean±SD	*P* value	Mean±SD	*P* value
Healthy controls	80 (100)	24.3±7.2	NS**	0.92±0.36	NS**
BPH	80 (100)	25.6±14.7		0.94±0.81	
Prostate cancer			<0.001		<0.005
Overall	120 (100)	37.2±20.3		1.43±1.37	
Clinical stage					
T1	22 (18)	27.4±14.9	<0.005	0.98±0.82	<0.05
T2	46 (38)	32.8±17.1		1.25±1.19	
T3	46 (38)	45.1±23.7		1.76±1.59	
T4	6 (6)	46.1±34.6		1.93±1.89	
Metastasis*					
Negative	71 (59)	32.4±15.7	<0.005	1.22±1.12	<0.05
Positive	49 (41)	44.2±23.9		1.78±1.45	
Lymph node metastasis					
Negative	108 (90)	36.2±17.9	NS	1.40±1.29	NS
Positive	12 (0)	46.2±31.8		1.70±1.66	
Bone metastasis					
Negative	74 (62)	33.1±16.8	<0.05	1.24±1.15	<0.05
Positive	46 (38)	43.8±26.3		1.73±1.39	
Gleason score					
>7	22 (18)	35.5±21.3	NS	1.38±1.30	NS
7	53 (44)	37.0±19.7		1.43±1.31	
7<	45 (38)	38.3±19.8		1.45±1.37	

*Either lymph node metastasis or bone metastasis, or both.
**Not significant.

Materials and Methods

Between January 1998 and December 2001, serum samples were obtained from 120 patients with prostate cancer (age 48 - 91 years, median 73) before any treatment and 80 patients with BPH (age 51-86 years, median 68). Diagnosis was confirmed histopathologically by transrectal needle biopsy under the guidance of transrectal ultrasonography (TRUS). Sera were also collected from 80 healthy male volunteers (age 49 - 83 years, median 66) who did not demonstrate any findings suggesting prostate cancer on clinical examinations, including digital rectal examination, TRUS and the measurement of serum PSA value. Of the 120 prostate cancer patients, 54 patients received hormonal therapy, while the remaining 66 underwent retropubic radical prostatectomy (RRP) and pelvic lymphadenectomy. Among the 66 surgically treated patients, 36 had pathologically confirmed organ-confined disease (pT1N0M0 or pT2N0M0) and 30 had non-organ-confined disease (pT3≤ and/or pN+). The pathological stages were determined according to the UICC (TNM) tumor stage classification system (23).

The concentrations of cathepsin B were determined using quantitative sandwich EIA kits for human pro-cathepsin B (R & D SYSTEMS, Minneapolis, MN, USA). A monoclonal antibody specific for cathepsin B was pre-coated onto a microplate. Samples were pipetted into the wells and any cathepsin B present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for cathepsin B was added to the wells. After washing to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of cathepsin B bound in the initial step. The color development was then stopped and the intensity of the color was measured using a microculture plate reader (Becton Dickinson Labware, Lincoln Park, NJ, USA). All analyses and calibrations were performed in duplicate. Each microtiter plate included a recombinant human cathepsin B standard for calibration. The blank value was subtracted from the duplicate readings for each standard and sample. A standard curve was created using Stat View 4.02 (Abacus Concepts Inc., Berkeley, CA, USA) by plotting the logarithm of the mean absorbance of each sample *versus* the sample concentration.

Prostate volume was determined by TRUS, as previously described (3). Briefly, the maximal anterior-posterior (AP) and transverse (TR) diameters were measured, and the maximal superior-interior (SI) diameter was measured from the base to the apex of the prostate in the midline sagittal plane. Prostate volume was calculated from the formula of a prolate ellipsoid: prostate volume = 0.52 x TR x AP x SI. Cathepsin B-D was defined as the serum cathepsin B level divided by the prostate volume.

Differences in the cathepsin B and cathepsin B-D levels between controls and prostate cancer patients were analyzed by Mann-Whitney *U*-test. Differences in the elevation of cathepsin B and cathepsin B-D levels were determined by Chi-square test. The cause-specific survival of prostate cancer patients was examined by the Kaplan-Meier method and differences were evaluated by the log-rank test. A difference with $p<0.05$ was considered significant.

Table II. *Elevation of serum cathepsin B and cathepsin B-D levels in patients with BPH and prostate cancer.*

	Number of subjects (%)	Elevation % Cathepsin B (%)	*P* value	Cathepsin B-D (%)	*P* value
BPH	80 (100)	7 (9)		4 (5)	
Prostate cancer			<0.001		<0.001
Overall	120 (100)	64 (53)		48 (40)	
Clinical stage					
T1	22 (18)	3 (14)	<0.001	1 (5)	<0.01
T2	46 (38)	21 (46)		10 (22)	
T3	46 (38)	36 (78)		33 (72)	
T4	6 (6)	4 (67)		4 (67)	
Metastasis*					
Negative	71 (59)	30 (42)	<0.01	21 (30)	<0.01
Positive	49 (41)	34 (69)		27 (56)	
Gleason score					
>7	22 (18)	11 (50)	NS	8 (36)	NS
7	53 (44)	27 (51)		19 (36)	
7<	45 (38)	26 (58)		21 (47)	

*Either lymph node metastasis or bone metastasis, or both.
**Not significant.

Table III. *Serum cathepsin B and cathepsin B-D levels in patients with BPH and prostate cancer who underwent radical prostatectomy.*

	Number of subjects	Cathepsin B (ng/ml) Mean±SD	*P* value	Cathepsin B-D (ng/ml^2) Mean±SD	*P* value	Elevation (%), *P* value Cathepsin B		Cathepsin B-D	
BPH	80	25.6±14.7		0.94±0.81		7(9)		4(5)	
Prostate cancer			NS**		NS		NS		NS
organ-confined*	36	30.1±18.1		1.06±0.90		8(22)		5(14)	
			<0.05		<0.05		0.01		<0.05
extraprostatic**	30	39.2±18.3		1.54±1.02		13(43)		12(40)	

*pT1N0M0 or pT2N0M0
**pT3 ≤ and/or pN+.
***Not significant.

Results

In healthy controls, the mean levels of cathepsin B and cathepsin B-D were 24.3±7.2 ng/ml (range 4.4 to 45.3 ng/ml) and 0.92±0.36 ng/ml^2 (range 0.23 to 1.53 ng/ml^2), respectively. As shown in Table I, there were no significant differences in cathepsin B and cathepsin B-D levels between healthy controls and patients with BPH; in contrast, the levels of cathepsin B and cathepsin B-D in patients with prostate cancer were significantly greater than those in patients with BPH. Furthermore, cathepsin B and cathepsin B-D levels in patients with a higher clinical stage (*i.e.*, T3 or T4) of prostate cancer were significantly greater than those in patients with a lower clinical stage (*i.e.*, T1 or T2) of prostate cancer. The levels of cathepsin B and cathepsin B-D in prostate cancer patients with metastasis were significantly greater than those in patients without metastasis. However, the levels of cathepsin B and cathepsin B-D in prostate cancer patients were not significantly associated with the Gleason score determined by biopsy findings.

In this series, the cut-off values for normal levels of serum cathepsin B and cathepsin B-D were determined by

the mean level in healthy controls plus 2 standard deviations; thus, the normal values for cathepsin B and cathepsin B-D were less than 38.7 ng/ml and 1.64 ng/ml[2], respectively. As shown in Table II, the elevation of cathepsin B and cathepsin B-D in prostate cancer patients was significantly higher that in patients with BPH. In addition, the differences in the elevation of cathepsin B and cathepsin B-D levels between prostate cancer patients with and without metastasis were also significant. However, there were no significant differences in elevation of cathepsin B and cathepsin B-D levels between prostate cancer patients according to the biopsy Gleason score.

The levels of serum cathepsin B and cathepsin B-D in 66 patients undergoing RRP were then analyzed according to the pathological stage. There were no significant differences in cathepsin B and cathepsin B-D levels between patients with BPH and organ-confined prostate cancer (*i.e.*, pT1N0M0 or pT2N0M0); however, differences in cathepsin B and cathepsin B-D levels between patients with organ-confined disease and extraprostatic disease (*i.e.*, pT3 ≤ or N+) were significant (Table III). Moreover, differences in the elevations of cathepsin B and cathepsin B-D between organ-confined disease and extraprostatic disease were also significant (Table III).

We finally compared the prognoses of patients with prostate cancer according to cathepsin B and cathepsin B-D levels. The cause-specific survival rate of prostate cancer patients with an elevated level of cathepsin B was not significantly different from that of patients with a normal level, and the difference in the cause-specific survival rate between prostate cancer patients with an elevated level of cathepsin B-D and patients with a normal level was also not significant (data not shown).

Discussion

Cathepsin B has been shown to be implicated in the degradation of basement membrane proteins and invasion of the adjunct stroma in several experimental tumor models (15-17), and the overexpression of cathepsin B shows a close association with disease progression in various kinds of human malignancy, including prostate cancer (9-12, 18-22). Furthermore, recent studies reported that measurement of serum levels of cathepsin B using sandwich EIA could serve as a useful prognostic marker for patients with certain types of malignant tumor (13, 14). To our knowledge, however, there have not been any studies investigating serum cathepsin B values in patients with prostate cancer; therefore, in the present study, serum levels of cathepsin B and cathepsin B-D were examined in healthy controls, BPH and prostate cancer patients, and an investigation into whether they could be used as predictors of disease progression and prognosis in prostate cancer patients was performed.

In the present series, serum levels of cathepsin B in patients with prostate cancer were found to be significantly higher than the levels in patients with BPH or healthy controls. A newly defined factor, cathepsin B-D, in patients with prostate cancer was also significantly higher than that in patients with BPH. Moreover, the values of these two factors paralleled the progression of prostate cancer. Considering these results, both cathepsin B and cathepsin B-D may be useful for differentiation between patients with prostate cancer and those with benign prostatic disease and they could be used to predict prostate cancer progression.

It is extremely important for the selection of therapeutic modalities to precisely distinguish prostate cancer patients with organ-confined disease from those with non-organ-confined disease preoperatively. To date, several methods have been introduced into clinical practice, including computerized tomography, magnetic resonance imaging, measurement of PSA and its related parameters and histological grading of biopsy specimens (3, 24); however, even if these methods are combined, it remains difficult to accurately identify patients with non-organ-confined disease. In this series, the elevation of either serum cathepsin B or cathepsin B-D in patients with extraprostatic disease was significantly higher than that in patients with organ-confined disease. These findings suggest that measurement of serum cathepsin B and cathepsin B-D, in addition to conventional parameters, may contribute to differentiating between patients with organ-confined and extraprostatic diseases.

As described above, serum cathepsin B and cathepsin B-D were well correlated to prostate cancer progression; however, there were no significant differences in cause-specific survival rates between patients with normal and elevated serum cathepsin B or cathepsin B-D. This discrepancy may have occured for several reasons, such as the comparatively small number of patients and short observation period, the initial good response to androgen withdrawal therapy in the majority of prostate cancer patients, and the collective analysis of patients who underwent radical prostatectomy and those who did not. Therefore, to definitively conclude that these two factors could be used as prognostic predictors for prostate cancer patients, it will be necessary to perform a prospective study including a larger number of patients with a longer follow-up period than the current study.

In conclusion, the present results suggest that the serum levels of cathepsin B and cathepsin B-D are closely associated with the extension of prostate cancer. Accordingly, measurement of serum cathepsin B and cathepsin B-D could serve as a useful practical adjunct to the conventional tools for differentiating between prostate cancer and BPH, as well as the prediction of prostate cancer progression.

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