Prognostic Potential of a PSA Complex in Sera of Prostate Cancer Patients With α 2-Macroglobulin Deficiency

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We previously reported on a number of cases of metastatic prostate cancer (PCa) in which serum α 2-macroglobulin (α 2M) levels were markedly decreased to less than 20 mg/dl (α 2M deficiency). In order to elucidate the relative proportions of free and a prostate-specific antigen (PSA) complex in PCa patients with α 2M deficiency, we have assessed serum α 2M and total PSA levels, and ratios of free PSA to total PSA (*F/T* ratios) at each stage of PCa. Moreover, the PSA reactivity profile was determined on fractionated serum specimens of PCa patients using high-performance liquid chromatography (HPLC) using a TSKG-3000 SWXL column. Measurement of α 2M concentration was performed by laser-nephelometry. PSA levels were determined by enzyme immunoassay,

free PSA by radioimmunoassay. In those PCa patients with α 2M deficiency, serum α 2M and *F/T* ratios were lower, whereas PSA levels were higher when compared with those PCa patients without α 2M deficiency ($P < 0.05$). PSA elution profiles on HPLC columns revealed two major peaks. The proportion of PSA-antichymotrypsin (PSA-ACT) increased, whereas the proportion of free PSA decreased in PCa patients with α 2M deficiency as compared with those PCa patients without α 2M deficiency. *F/T* ratios were significantly lower in PCa patients with α 2M deficiency than in those PCa patients without α 2M deficiency. PSA-ACT and *F/T* ratio may be useful for monitoring bone metastasis in PCa. *J. Clin. Lab. Anal.* 22:302–306, 2008. © 2008 Wiley-Liss, Inc.

Key words: prostate-specific antigen; α 1-antichymotrypsin; α 2-macroglobulin deficiency

INTRODUCTION

Prostate-specific antigen (PSA) is a 33 kDa glycoprotein secreted by prostatic glandular epithelial cells. PSA is thought to be a serine protease similar to endocrine kallikrein and serves to liquefy seminal coagulum (1,2). This enzyme is widely used as a specific marker for diagnosis, prognosis, and monitoring of therapeutic effects in prostate cancer (PCa) patients (3–5). PSA is present as a free molecule and PSA complexed with α 1-antichymotrypsin (ACT) or α 2-macroglobulin (α 2M) in the blood (6,7). However, PSA complexed to α 2M is not detected in current commercial assay for PSA (8,9). It is recognized that the relative proportions of free and PSA complex differ between PCa and benign prostate hypertrophy (BPH), and that the ratio of free PSA to total PSA (*F/T* ratio) is useful for differential diagnosis of localized PCa and BPH (10,11).

α 2M is a 720 kDa glycoprotein, and is the most abundant protease inhibitor in blood. α 2M inhibits many protease activities and is involved in coagulation, fibrinolytic action, and inflammatory reactions in vivo. Furthermore, another important biological function of α 2M may be as a carrier protein for interleukin-6 and growth hormone (12,13).

Serum α 2M levels show slight increases in a variety of different disorders, such as nephrotic syndrome, chronic

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inflammatory diseases, or malignancy. On the other hand, decreases of α 2M levels are known only in very few disorders, such as disseminated intravascular coagulation (DIC) or hematologic malignancy. We previously reported on a number of cases of metastatic PCa in which serum α 2M levels were markedly decreased to less than 20 mg/dl (α 2M deficiency), whereas serum PSA levels were remarkably increased (14,15). These cases were not complicated by DIC.

However, there are no reports on the relative proportions of free and PSA complex in serum of PCa patients with α 2M deficiency. The aim of this study, therefore, was to obtain this information. We have assessed serum α 2M and total PSA levels, and *F/T* ratios at each stage of PCa. Moreover, PSA reactivity was determined in serum specimens fractionated by high-performance liquid chromatography (HPLC) of PCa patients.

MATERIALS AND METHODS

Patient Population

There were 96 untreated subjects who participated in this study. Of these, 10 were controls and 86 were diagnosed with prostatic diseases at the Kitasato University Hospital and cooperating hospitals. Of the latter group, 32 patients had BPH (mean age 68.2 years, range 52–80 years) and 54 patients had PCa (stages T1–T2: 14 cases; stage T3: 8 cases; stage M1b: 32 cases; mean age 69.3 years, range 56–78 years). None of the cases had DIC, which was screened by the measurement of fibrinogen, fibrinogen degradation products, and D-dimer. Ten healthy adult males were enrolled in the control group (mean age 62.4 years, range 54–70 years). Histology was confirmed by six-sextant biopsy and/or transurethral resection in all cases. PCa was clinically staged following the tumor, node, metastasis classification (16). Briefly, stage T1 is defined as tumor not clinically recognizable and identifiable only by histological examination of prostatic tissue. Stage T2 tumors are palpable but confined within the prostate. Stage T3 tumors are palpable and extend through the prostatic

capsule with unilateral or bilateral extension. M1 stage is defined by the presence of distant metastasis and M1b by bone metastases. The serum samples were stored at -80°C until use.

Laboratory Methods

Measurement of α 2M concentration was performed by laser-nephelometry (Behring-nephelometer-analyzer, Behring, Somerville, NJ). PSA levels were determined by enzyme immunoassay (AIA-600, TOSOH, Tokyo, Japan), free PSA by radioimmunoassay (WALLAC-1460, Pharmacia, Uppsala, Sweden). Fractionation of serum samples was performed by HPLC using a TSKG-3000 SWXL (TOSOH) column and PSA levels in each of the fractions were measured. Flow rate was 1 ml/min and fractions of 0.5 ml volume were collected. The column was calibrated using 20 mM phosphate buffer (pH 7.5). Bone metastasis in PCa patients was diagnosed by bone scintigraphy using $^{99\text{m}}\text{Tc}$ -labeled methylene diphosphonate. All PCa patients with stage M1b showed multiple sites of isotope accumulation.

Statistical Analysis

Statistical analysis was performed with the nonparametric Mann-Whitney *U*-test. Statistical significance was defined as $P < 0.05$.

RESULTS

The Concentration (Mean $\pm$ SD) of α 2M and PSA in Serum

Table 1 gives the concentrations of α 2M and PSA in the sera of healthy controls, BPH, and PCa. There were no significant differences in serum levels of α 2M and PSA between healthy controls and BPH or PCa patients in stages T1–T2. Serum α 2M levels tended to decrease, and serum PSA levels increased with the progression of PCa. In invasive (T3) and metastatic (M1b) PCa patients, PSA levels were significantly higher than in those of healthy controls and BPH ($P < 0.05$).

TABLE 1. Concentration (Mean $\pm$ SD) of α 2-Macroglobulin (α 2M) and Total Prostate-Specific Antigen (PSA) in Sera of Healthy Controls, Benign Prostate Hypertrophy (BPH), and Prostate Cancer Patients

	Healthy controls (<i>n</i> = 10)	BPH (<i>n</i> = 32)	Prostate cancer		
			Stages T1, T2 (<i>n</i> = 14)	Stage T3 (<i>n</i> = 8)	Stage M1b (<i>n</i> = 32)
α 2M (mg/dl)	151.7 $\pm$ 25.30	208.40 $\pm$ 59.80	235.40 $\pm$ 75.20	184.80 $\pm$ 43.70	112.10 $\pm$ 91.40
PSA (ng/ml)	1.58 $\pm$ 0.80	4.90 $\pm$ 4.70	16.60 $\pm$ 17.70	44.0 $\pm$ 28.50 [†]	985.0 $\pm$ 904.90 [†]

* $P < 0.05$, healthy controls vs. BPH or prostate cancer.

[†] $P < 0.05$, BPH vs. prostate cancer.

TABLE 2. Concentration (Mean±SD) of α2-Macroglobulin (α2M), Total Prostate-Specific Antigen (PSA) and Free PSA/Total PSA (F/T) Ratio in Sera of Healthy Controls and Prostate Cancer Patients (Stage M1b)

	Healthy controls (n = 10)	Prostate cancer (stage M1b)	
		Without α2M deficiency (n = 23)	With α2M deficiency (n = 9)
α2M (mg/dl)	151.7 ± 25.30	173 ± 58.20	11.90 ± 6.10 ^{*†}
PSA (ng/ml)	1.58 ± 0.80	659 ± 441.50 [*]	5,472.5 ± 2,784.6 ^{*†}
F/T (ratio)	32.10 ± 19.30	0.32 ± 0.18 [*]	0.09 ± 0.03 ^{*†}

^{*}P<0.05, healthy controls vs. prostate cancer without α2 deficiency or with α2 deficiency.

[†]P<0.05, prostate cancer without α2 deficiency vs. prostate cancer with α2 deficiency.

Fig. 1. PSA elution profiles in sera of prostate cancer patients (stage M1b without α2M deficiency) on a TSKG-3000 SWXL HPLC column. PSA, prostate-specific antigen; α2M, α2-macroglobulin.

Fig. 2. PSA elution profiles in sera of prostate cancer patients (stage M1b with α2M deficiency) on a TSKG-3000 SWXL HPLC column. PSA, prostate-specific antigen; α2M, α2-macroglobulin.

The Concentration (Mean±SD) of α2M, PSA, and free PSA/total PSA (F/T) ratio

Table 2 gives concentrations of α2M, PSA, and free PSA/total PSA (F/T) in the sera of healthy controls and PCa (M1b) patients. PCa patients with and without α2M deficiency were distinguished by analyses of PSA, α2M, and F/T ratio. PCa patients with α2M deficiency had markedly increased serum PSA levels and multiple

bone metastases. Also, serum α2M levels and F/T ratios were significantly lower than those in healthy controls and in those PCa patients without α2M deficiency (P<0.05).

The PSA Elution Profiles in Serum of PCa

Figures 1 and 2 show PSA elution profiles of sera from PCa (M1b) patients without and with α2M

deficiency on TSKG-3000 SWXL HPLC columns. The general elution pattern, showing two peaks of activity, was similar in both groups. The first peak at approximately 100 kd corresponded to a PSA-ACT complex, and a second peak at approximately 30 kd represented free PSA. The PSA-ACT complex in the first peak was higher in the sera of PCa patients with $\alpha 2M$ deficiency.

DISCUSSION

PSA is a serine protease secreted by prostate epithelial cells, and is useful as a specific tumor marker for PCa. In blood, PSA is present as a free molecule and PSA complexed with $\alpha 1$ -ACT or $\alpha 2M$. Complex PSA is predominantly bound to $\alpha 1$ -ACT, and that PSA complexed to $\alpha 2M$ is not detected in current commercial analysis for PSA (8,9). The *F/T* ratio has been used for the differential diagnosis of localized PCa and BPH (10,11). On the other hand, $\alpha 2M$ is the most abundant protease inhibitor in blood, and inhibits many protease activities related to fibrinolytic action and inflammatory reactions. It has also been reported that $\alpha 2M$ can inhibit the activity of proteases such as matrix metalloproteinases, and thus may inhibit the ability of cancers to infiltrate and metastasize (17). The $\alpha 2M$ -protease complex retains a weak enzyme activity, but the residual protease activity is rapidly eliminated from blood by the reticuloendothelial system (18,19). We have previously reported that the relative proportion of free and complex PSA is different in PCa and BPH patients, and that PSA-ACT levels increase with the progression of PCa. We also demonstrated that the *F/T* ratio is useful to differentiate localized PCa from BPH in patients with intermediate levels of PSA (4–10 ng/ml) (20).

In this study, we have assessed levels of serum $\alpha 2M$ and total PSA, and *F/T* ratios in patients with PCa. Moreover, the PSA reactivity profile was determined on fractionated serum specimens of PCa patients using HPLC. Therefore, the relative proportion of free and complex PSA was determined in PCa patients with $\alpha 2M$ deficiency. PSA levels in serum increased with the progression of PCa, and were significantly higher in invasive and metastatic PCa patients than in those of healthy controls and BPH. Similar results have been reported by others (3–5). We also demonstrated that serum PSA levels of PCa patients with $\alpha 2M$ deficiency were significantly higher than in those PCa patients without $\alpha 2M$ deficiency. The *F/T* ratios of PCa patients with $\alpha 2M$ deficiency were significantly lower than in those PCa patients without $\alpha 2M$ deficiency. It appears, thus, that patients with advanced PCa are likely to have multiple bone metastases and elevated serum PSA combined with $\alpha 2M$ deficiency.

On the other hand, serum $\alpha 2M$ levels tended to decrease with the progression of PCa. Serum $\alpha 2M$ levels tended to decrease markedly (< 20 mg/dl) in cases where serum PSA levels showed striking increases ($> 2,000$ mg/dl). The decrease in serum $\alpha 2M$ levels may be brought about by the reticuloendothelial system rapidly removing many $\alpha 2M$ -PSA complexes from the blood in PCa patients with extremely high levels of serum PSA (18,19).

Using HPLC, we found in concurrence with others that the PSA reactivity profile of fractionated serum specimens of PCa patients was mainly divided into two peaks (8,21). The first peak at approximately 100 kd corresponded to a PSA-ACT complex and the second peak at approximately 30 kd represented free PSA. The first peak of PSA-ACT complex was higher in PCa patients with $\alpha 2M$ deficiency than in those PCa patients without $\alpha 2M$ deficiency. It is showed that serum $\alpha 1$ -ACT levels were higher in patients with PCa than in those without PCa (22,23). Kikuchi et al. have also reported that PSA-ACT is useful for identifying patients with a low probability of having bone metastasis (24). It was thought that serum levels of $\alpha 1$ -ACT, a marker of inflammation, increased in PCa patients with $\alpha 2M$ deficiency than in those PCa patients without $\alpha 2M$ deficiency.

In conclusion, the proportion of PSA-ACT increased and the proportion of free PSA decreased in PCa patients with $\alpha 2M$ deficiency compared with PCa patients without $\alpha 2M$ deficiency. *F/T* ratios were significantly lower in PCa patients with $\alpha 2M$ deficiency than in those PCa patients without $\alpha 2M$ deficiency. PSA-ACT and *F/T* ratio may be useful for monitoring of bone metastasis in PCa.

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