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Original Paper

Soluble CD44 Molecules in Serum of Patients with Prostate Cancer and Benign Prostatic Hyperplasia

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Recent studies suggest that expression of CD44 splice variants are of prognostic significance for a variety of neoplasias. It was the aim of this study to investigate whether any correlation exists between the concentration of soluble CD44 molecules in serum (CD44 standard form and CD44 splice variants v5 and v6) and the prostate cancer stage. Serum levels of these soluble CD44 isoforms were measured by ELISA tests specific for these proteins in controls (n = 30), patients with benign prostatic hyperplasia (BPH; n = 30), with prostate cancer without metastasis (T1,2,3pN0M0; n = 30) and with locally advanced prostate cancer and/or metastatic disease (T3,4pN1,2M1; n = 19). sCD44std and sCD44v6 concentrations were not significantly different among the four groups studied, with few patients' levels outside the central 95% reference intervals. The mean sCD44v5 concentrations of both prostate cancer and BPH patients were significantly lower than those of the controls. There was no significant difference between the soluble CD44 concentrations of the two groups of prostate cancer patients studied. In contrast to results observed in other carcinomas, the determination of soluble CD44 proteins in serum is not suitable for providing additional prognostic information on patients with prostate cancer.

Key words: carcinoma immunology, cell adhesion molecules, prostate cancer, BPH, tumour marker
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INTRODUCTION

NUMEROUS ADHESION molecules are expressed by tumour cells and a variety of adhesive interactions are mediated by them [1, 2]. They act as both positive and negative modulators of the invasive and metastatic processes. One of these interesting proteins is CD44, a transmembrane glycoprotein functioning as the principal receptor for the glycosaminoglycan hyaluronate and playing an important role in cell–extracellular matrix contacts [3]. CD44 proteins comprise a family of very heterogeneous proteins which are encoded by a gene composed of 19 exons [4, 5]. In general, two groups of CD44 molecules are distinguished: a standard form of 80–90 kDa, the so-called CD44std form, encoded by the first five and last five exons and found on the surface of haematopoietic, specific glandular and fibroblastoid cells; and the group of CD44 variants (CD44v) of more than 110 kDa [6]. These larger CD44 proteins are in part due to post-translational modification and in part due to the additional nine exons which are alternatively spliced. The CD44v proteins consist of the same extracellular N-terminal sequence, the transmembrane and cytoplasmic parts as the CD44std form, but they contain additional amino acid sequences encoded by the variant exons. The variant forms are weakly expressed on normal epithelial cells.

In tumour cells, an overproduction of large CD44v proteins by enhanced transcription of alternatively spliced variant CD44v mRNA has generally been observed [7]. Increased expression of CD44v proteins correlates with advanced stages of human breast, uterine, cervical and colon cancer, and has been found in human carcinoma lines from lung, breast and colon [8–12]. First results in patients with gastric and colon cancer have shown that these changes on the cellular surface are reflected by increased concentrations of soluble CD44 in serum [13].

The determination of CD44 proteins in serum has been suggested as a simple, non-invasive tool in cancer diagnostics and in monitoring the efficacy of treatment [13]. Therefore, results of other tumours are urgently needed to test this hypothesis. Since assays for the determination of soluble CD44std, CD44v5 and CD44v6 proteins in serum have

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recently been introduced, it was the objective of this study to evaluate the potential usefulness of those assays in patients with prostate cancer.

PATIENTS AND METHODS

Patients and samples

A total of 109 men were studied, including a control group of 30 healthy men (median age 54 years, range 38–79), 30 patients with benign prostate hyperplasia (BPH) (median age 71, range 57–85) and 49 with prostate cancer (median age 66 years, range 45–78). All samples were taken before initiation of therapy. The diagnosis of BPH was based on histological analysis of tissue obtained by transurethral resection of the prostate. The diagnosis of prostate cancer was established cytopathologically by transrectal fine needle biopsy or histopathologically by microscopic examination of prostatic specimens after biopsy or prostatectomy. Clinical staging was made according to the TNM system by digital rectal examination, transrectal ultrasonography and determination of prostate-specific antigen (PSA) including surgical assessment of lymph node status. Of the 49 patients with prostate cancer, 30 (median age 66 years, range 48–74) had prostate cancer without metastasis (T1,2,3pN0M0) and 19 (median age 66 years, range 45–78) had prostate cancer with metastasis (T3,4pN1,2M1). 11 of these 19 patients had metastases in regional lymph nodes and 9 had distant metastases.

Blood was collected before digital rectal examination and instrumentation. The serum was obtained by centrifugation at 1500 times gravity for 10 min after allowing the blood to clot for 1 h at room temperature. The serum samples were analysed either the same day or stored at −70°C before analysis.

sCD44 ELISA assays

The sCD44 tests (Bender MedSystems, Vienna, Austria) are enzyme-linked immunosorbent assays (ELISA) based on the sandwich principle using two highly specific murine monoclonal antibodies for coating the solid phase (microtiterplate) and for the tracer horseradish peroxidase. According to the data given by the manufacturer, the respective assays specifically recognise the corresponding sCD44 molecules. The antibodies and their specificity have been previously described [8, 9]. There is no detectable crossreactivity with circulating factors of the immune system (e.g. TNF-α, TN-β) and with CD44 polypeptides lacking the protein sequence encoded by the corresponding exons. The limits of detection for sCD44std, sCD44v5 and sCD44v6, respectively, defined as the corresponding concentrations located 3 S.D.s above the measured average blanks, were 0.07, 0.22 and 0.09 μ/l, respectively.

One hundred microlitres of the standard/control/sample and 50 μl of the tracer antibody are added to the wells and incubated for 3 h at room temperature on a rotator. The tracer antibody and the antibody of the microwell react simultaneously with CD44 present in standards and samples. Unbound CD44 and tracer antibody are removed by three washing steps. Then, tetramethyl-benzidine as substrate reactive with the horseradish peroxidase is added, and the tracer–CD44 complex bound to the microwell reacts by the peroxidase reaction with the substrate. The reaction is terminated by addition of sulphuric acid and the absorbance is measured on a microplate reader at 450 nm. The concentrations were calculated by means of a standard curve using the cubic spline procedure. All tests were performed in duplicates and average values were used for further calculations.

PSA was measured by the Axsym test (Abbott Laboratories, Abbott Park, Illinois, U.S.A.). All patients had their PSA determined.

Statistical analysis

Statistical calculations were performed by the statistical package Statgraphics, version 5.01 (Statistical Graphics Corp., Rockville, Maryland, U.S.A.). Mann–Whitney *U*-test with independent samples, correlation coefficient according to Spearman, the ANOVA analysis of variance and the distribution fitting procedure as Kolmogorov–Smirnov test were used. The central 95% reference intervals in the control group were calculated according to the IFCC-recommended procedure [14]. $P < 0.05$ was considered statistically significant.

RESULTS

Serum concentrations of sCD44std, sCD44v5 and sCD44v6 measured in 30 healthy men of ages comparable to those of the patients included in this study were not age-dependent (r_s = 0.08, −0.19 and −0.13, respectively). sCD44std did not correlate to the two sCD44 variant proteins (r_s = 0.15 and 0.27, respectively) but sCD44v5 and sCD44v6 were strongly correlated (r_s = 0.72). We found normal distributions of all sCD44 proteins, sCD44std showing higher concentrations (mean ± S.D., 501 ± 87 μg/l) than sCD44v5 (54.1 ± 22.7 μg/l) and sCD44v6 (179 ± 64 μg/l). Therefore, we calculated the upper and lower reference limits as the mean +1.96 S.D. and mean −1.96 S.D., respectively, using the central 95% reference interval as the basis [14]. The corresponding limits, which are also indicated in Figure 1, were 672 and 330 μg/l for sCD44std, 100 and 10 μg/l for sCD44v5, and 304 and 54 μg/l for sCD44v6, respectively.

Figure 1 shows the behaviour of the three sCD44 proteins in serum of patients with BPH and prostate cancer. Patients with prostate cancer were subdivided into two groups, patients without metastasis and patients with distant metastasis and/or metastasis in regional lymph nodes. It was evident that sCD44std and sCD44v6 concentrations were not different among the four groups studied (ANOVA; *U*-test); the concentrations observed in patients were not different from those in the control group. Only a few patients were outside the reference limits (Figure 1). The mean sCD44v5 concentrations in BPH and prostate cancer patients were significantly lower than those measured in the control group, whereas almost all individual data were within the reference limits. There was no significant difference in sCD44v5 concentrations among the three groups of patients. All sCD44 values in patients without metastases were not significantly different from corresponding values of patients with metastatic prostate cancer (Figure 1a–c).

There was no significant correlation between sCD44 proteins and the PSA values. PSA values of healthy men (median concentration 0.98, lower quartile 0.65 and upper quartile 1.54 μg/l), BPH patients (5.9; 2.8 and 8.3 μg/l) and prostate cancer patients without metastasis (8.9; 7.1 and 16.3 μg/l), and more locally advanced and/or metastatic disease (40; 16.5 and 252 μg/l) were clearly different. The Axsym assay used in this study defines 4 μg/l as the upper normal limit. However, the instruction sheet of the assay stresses that approximately 30% of patients with BPH have PSA concentrations above 4 μg/l. In our study, the diagnosis of BPH was based in all cases on histological analysis of tissue obtained by transur-

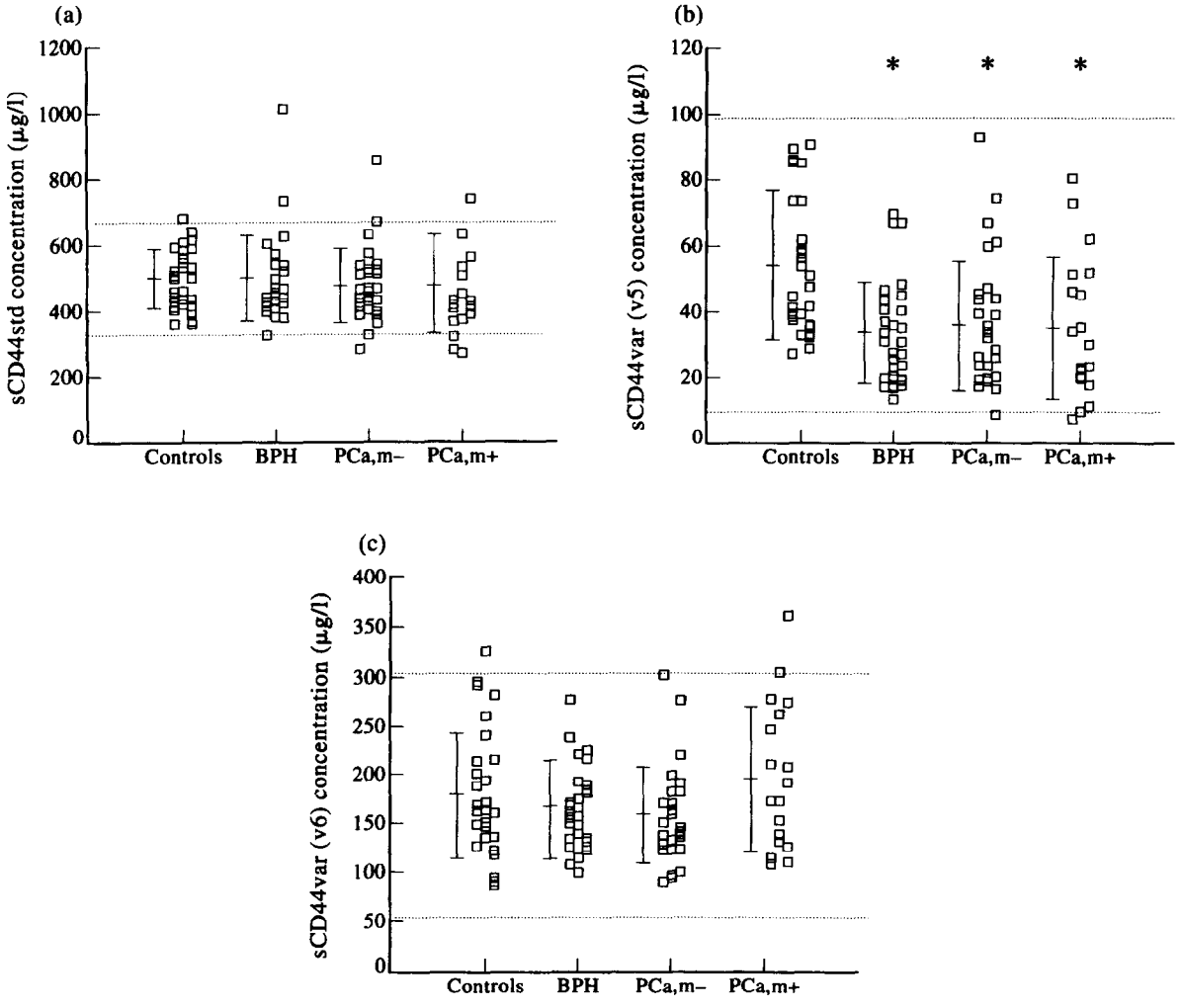

Figure 1. Serum concentrations of (a) sCD44std, (b) sCD44v5 and (c) sCD44v6 in controls, patients with benign prostate hyperplasia (BPH) and with prostate cancer with (PCa, m+) and without metastasis (PCa, m−). Values are expressed as individual values and as arithmetic means ± S.D. Significance in comparison to controls: *, at least $P < 0.05$.

ethral resection of the prostate and there was no indication of an early prostate cancer.

DISCUSSION

Determination of soluble CD44 fragments in body fluids has been suggested as a promising non-invasive method to assess tumour burden and metastases in patients with malignant diseases [13]. Until now, there are very few reports on soluble CD44 molecules and their clinical significance because methods for determining soluble CD44 molecules have only recently been introduced [13, 15, 16]. The specificity of antibodies used in the assays to detect the different isoforms of the CD44 in serum has been validated by different methods including PCR technique [8, 9, 17]. No interference from circulating circulating factors of the immune system (e.g. TNF-α, TN-β) and with CD44 polypeptides lacking the protein sequence encoded by the corresponding exons has been observed.

Increased standard and variant sCD44 proteins have been found in serum of patients with colon, gastric and breast cancer when the same assays were used as in our study or when similar tests were applied [13, 15, 16]. Since surgical tumour resection significantly reduced the sCD44 concentration in serum, it was assumed that the soluble CD44 in the blood most likely originated from tumour cells [13]. However, our results showed that all the sCD44 isoforms investigated in this study, the standard form and the two variants sCD44v5 and sCD44v6, occurred in serum of healthy men in measurable concentrations. Concentrations varied between isoforms with the highest concentrations found in the standard form. However, a comparison of the findings in the patient groups and in healthy persons gave no evidence of clinical validity of sCD44 determination in patients with prostate cancer. We conclude from our results that alterations observed in patients with other tumours such as gastric or colon carcinomas could not be detected in patients with prostate cancer, even those with metastatic disease. Recently, similar findings in cervical cancer patients were observed [18]. Serum CD44 concentrations showed no significant correlation with the absence or presence of cancer.

Expression of CD44 variants is not an obligatory requirement for metastasis formation, but there is strong evidence that increased expression of the CD44 proteins on tumour cells correlates with tumour development [7, 13]. The expression pattern of CD44 has been studied in various tissues under normal and cancerous conditions using polymerase chain reaction or monoclonal antibodies specific for various CD44 isoforms [8–11, 17, 19]. It is possible that these two

techniques may yield discordant results [13, 17]. However, a comparison of results obtained by immunohistochemistry and reverse transcriptase–polymerase chain reaction (RT-PCR) technique showed that there was a good agreement between most of the data [17]. Reasons for these discrepancies are not known. Until now, the behaviour of CD44 expression in prostate tissue has not been studied as has been done in other tissues. Using the polymerase chain reaction, increased CD44 expressions were recently observed in human prostate cell lines [20]. PC3 and DU145 cells, which are tumorigenic and metastatic in nude mice, have increased expressions of standard and variant CD44, whereas changes of this kind have not been detected in non-metastatic LNCaP cells. From these results, a relationship between the state of cellular dedifferentiation and the activation of CD44 expression in prostate cancer was hypothesised [20]. Such speculation seems to be doubtful as long as the true expression of CD44 has not been demonstrated in normal and cancerous prostate tissue. The almost identical concentrations of sCD44 proteins found in our prostate cancer patients compared to healthy men and BPH patients, respectively, could be attributed to two causes, either the expression of CD44 proteins in the prostate is not typically changed by cancer or the existing cellular alteration is not reflected in serum. Both causes might contribute separately or together.

Overexpression of variant or standard CD44 can therefore not be used as a general indicator of neoplasia [21]. Penno and associates [19] found an irregular expression of CD44 in various types of lung tumours. For example, non-small cell lung cancer was characterised by increased CD44std, which was not found in patients with small lung cancer. This result and our observation that decreased sCD44v5 concentrations occurred in both BPH and PCa patients are inconsistent with the first assumption.

In summary, we conclude that determination of soluble CD44 proteins in serum is not suited for staging patients with prostate cancer. Experiments are now in progress to investigate other urogenital tumours.

1. Stetler-Stevenson WG, Aznavoorian S, Liotta LA. Tumor cell interaction with extracellular matrix during invasion and metastasis. *Ann Rev Cell Biol* 1993, **9**, 541–573.
2. Albelda SM. Biology of disease. Role of integrins and other cell adhesion molecules in tumor progression and metastasis. *Lab Invest* 1993, **68**, 4–17.
3. Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal surface receptor for hyaluronate. *Cell* 1990, **61**, 1303–1313.
4. Screaton GR, Bell MV, Bell JI, Jackson DG. The identification of a new alternative exon with highly restricted tissue expression in transcripts encoding the mouse Pgp-1 (CD44) homing receptor. Comparison of all 10 variable exons between mouse, human, and rat. *J Biol Chem* 1993, **268**, 12,235–12,238.
5. Tölg C, Hofmann M, Herrlich P, Ponta H. Splicing choice from ten variant exons establishes CD44 variability. *Nucleic Acids Res* 1993, **21**, 1225–1229.
6. Stamenkovic I, Aruffo A, Aminot M, Seed B. The hematopoietic and epithelial forms of CD44 are distinct polypeptides with different adhesion potentials for hyaluronate bearing cells. *EMBO J* 1991, **10**, 343–348.
7. Ponta H, Sleeman J, Herrlich P. Tumor metastasis formation: cell-surface proteins confer metastasis-promoting or -suppressing properties. *Biochim Biophys Acta* 1994, **1198**, 1–10.
8. Heider KH, Hofmann M, Hors E, *et al.* A human homologue of the rat metastatic-associated variant of CD44 is expressed in colorectal carcinomas and adenomatous polyps. *J Cell Biol* 1993, **120**, 227–233.
9. Koopman G, Heider KH, Horst E, *et al.* Activated human lymphocytes and aggressive non-Hodgkin's lymphomas express a homologue of the rat metastasis-associated variant of CD44. *J Exp Med* 1993, **177**, 897–904.
10. Dall P, Heider KH, Hekele A, *et al.* Surface protein expression and messenger RNA-splicing analysis of CD44 in uterine cervical cancer and normal cervical epithelium. *Cancer Res* 1994, **54**, 3337–3341.
11. Wielenga VJM, Heider KH, Johan G, *et al.* Expression of CD44 variant proteins in human colorectal cancer is related to tumor progression *Cancer Res* 1991, **51**, 5292–5297.
12. Hofmann M, Rudy W, Zöller M, *et al.* CD44 splice variants confer metastatic behavior in rats: homologous sequences are expressed in human tumor cell lines. *Cancer Res* 1991, **51**, 5292–5297.
13. Guo Y, Liu G, Wang X, *et al.* Potential use of soluble CD44 in serum as indicator of tumor burden and metastasis in patients with gastric or colon cancer. *Cancer Res* 1994, **54**, 422–426.
14. Solberg HE. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. *J Clin Chem Clin Biochem* 1987, **25**, 645–656.
15. Steger G, Mader R, Djavanmard M, Gnant M, Jakesz R. sCD44v5 bei colorektalem Karzinom. CD44-Symposium, 9 June 1994, Vienna.
16. Kitt EM, Ruckser R, Huber K, *et al.* sCD44 Standard und die Varianten (v5) and (v6) bei Mammakarzinom. *Lab Med* 1995, **19**, 103.
17. Dall P, Heider KH, Sinn HP. Comparison of immunohistochemistry and RT-PCR for detection of CD44v-expression, a new prognostic factor in human breast cancer. *Int J Cancer* 1995, **60**, 471–477.
18. Kainz C, Tempfer C, Winkler S, Sliutz G, Koelbl H, Reinthaller A. Serum CD44 splice variants in cervical cancer patients. *Cancer Lett* 1995, **90**, 231–234.
19. Penno MB, August JT, Baylin SB, *et al.* Expression of CD44 in human lung tumors. *Cancer Res* 1994, **54**, 1381–1387.
20. Liu AY. Expression of CD44 in prostate cancer cells. *Cancer Lett* 194, **76**, 63–69.
21. Matsumara Y, Tarin D. Significance of CD44 gene products for cancer diagnosis and disease evaluation. *Lancet* 1992, **340**, 1053–1058.

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