ORIGINAL ARTICLE

High Concentration of Soluble Form of Vascular Endothelial Cadherin in Sera of Patients with Prostate Cancer

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Abstract

Background: For many years, prostate-specific antigen (PSA) was used to screen prostate cancer (PC) patients. However, recent controversial findings have cast doubt on the accuracy of this biomarker for diagnostic and prognostic purposes, and have stimulated the search for new candidates. This study was conducted to determine the capability of a soluble adhesion molecule known as soluble vascular endothelial cadherin (sVE-cadherin) or CD144 to distinguish prostate cancer or benign prostate hyperplasia (BPH) patients from healthy individuals.

Methods: Patients recently diagnosed as having PC (N=35) or BPH (N=35) and age-matched controls (N=30) were study enrolled. The concentration of sVE-cadherin and PSA was measured by ELISA. Gleason score in patients with PC was determined by pathological examination of tumor biopsies.

Results: The concentration of sVE-cadherin in the serum of patients with PC and BPH was significantly higher than that in the healthy men. No association was found between the concentration of this soluble adhesion molecule and PSA values. Moreover, concentrations of sVE-cadherin did not correlate with Gleason scores in patients with PC.

Conclusion: The high concentration of sVE-cadherin in our patients suggests that this bio-marker is a potentially useful tool to identify high-risk patients. However, further research in patients with PC and other pathological conditions is needed to support the efficacy of this molecule in PC screening.

Keywords: Prostate cancer; Soluble vascular endothelial cadherin; PSA

Introduction

The identification of prostate-specific antigen (PSA) revolutionized prostate cancer (PC) screening and has positively impacted PC detection and treatment in the past two decades. However, the emergence of controversial data has undermined its prognostic significance in recent years. Even derivatives of total PSA values such as PSA density (PSAD), PSAD of the transition zone, age-specific values and assessment of various isoforms of PSA, which have been used by different investigators, have not improved the performance of this biomarker in PC screening and epidemiology.[1] It is now believed that there is no true PSA cutoff point for identifying PC risk, as in a significant number of men who actually have prostate cancer, PSA values less than 4.0 ng/ml have been found.[2]

Prostate-specific antigen, an androgen-regulated serine protease of the tissue kallikrein family, can be produced by all types of normal, hyperplasic or cancerous prostatic cells, with highest levels found in the transitional zone of the prostate. Moreover, PSA concentration can also be elevated in nonmalignant disorders such as benign prostatic hyperplasia (BPH), infections or chronic inflammation.[3-5] The relationship of PSA levels with tumor grade is not well understood since it has been suggested that total PSA

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expression decreases with higher Gleason score.[6] Therefore, PSA can no longer be considered as a classical tumor marker for prostate cancer, and novel approaches with new molecular targets are needed to detect PC and foretell the course of the malignancy.

Of the plethora molecules with different roles, the adhesion molecule known as vascular endothelial cadherins (VE-cadherin) has unique features. Also known as CD144 and cadherin-5, VE-cadherin is an angiogenesis-related adhesion molecule.[7] This molecule is a 125-KDa single-pass transmembrane glycoprotein that associates as *cis* dimers via the extracellular domain on the cell surface to promote intercellular homophilic adherence junctions.[8] The expression of VE-cadherin complex on the cell surface is thought to be variable and dynamic depending on the functional state of the cell. This cadherin mainly functions to maintain and restore endothelial integrity, and is involved in determining the vascular architecture. Inactivation of the VE-cadherin gene in transgenic mice leads to embryonic mortality due to severe vasculogenic defects.[9,10] The extracellular part of VE-cadherin can be cleaved by proteolytic enzymes to give rise to the soluble form, which is detectable in the serum and is referred to as soluble cell adhesion molecule.[11]

Given the importance of angiogenesis for tumor survival and aggressiveness, in this study we measured the concentration of serum sVE-cadherin and PSA in healthy individuals, patients with BPH and patients with PC before therapy, and searched for correlations between these values, and between each value and disease score in patients with PC.

Materials and Methods

We studied 35 patients with recently diagnosed prostate cancer (mean age=71.7±7.7 years) and 35 patients with BPH (mean age=71.5±7.5 years). The control group consisted of 30 men (mean age=66±8.9 years) with no significant prostate or urinary tract problems and no history of severe metabolic disorders. None of the patients or healthy control participants had apparent severe infection or autoimmune disease. All the patients and normal subjects gave their written informed consent prior to participation in the study.

Blood samples for sVE-cadherin and PSA measurement were obtained from peripheral blood prior to therapy for the disease. From blood samples, the sera were separated by centrifugation at 700 g for 10 min, and serum aliquots were stored at –70 °C until the time of assay.

Serum concentration of sVE-cadherin was measured by ELISA (Bender Medsystem, Vienna, Austria) according to the manufacturer's instructions with a sensitivity of 0.15 ng/ml. Prostate-specific antigen concentration was measured by radioimmunoassay (Spectra, Finland) based on the manufacture's instructions. All analyses and calibrations were carried out in duplicate, and intra- and inter-assay variations were within the range given by the manufacturers.

Statistical analysis was performed with SPSS software (version 11.5, Chicago, IL, USA) and Prism Graphpad software. All the results are expressed as the mean±standard deviation. Non-parametric Kruskal-Wallis test was used to compare the values between different groups. *P* values <0.05 were considered statistically significant.

Results

Prostate cancer was confirmed by pathological examination of prostate biopsies and scored according to the Gleason system from 4 to 10 (mean=7.11±1.64). Of these, almost 37% had a Gleason score of 8-10, and nearly 50% had a less severe score of 6-7. In the rest of the patients, the disease score was 4-5 (Table 1).

Table 1: Frequency of patients with prostate cancer at different Gleason score

Gleason Score	No (%)
4	2 (5.7)
5	2 (5.7)
6	10 (28.6)
7	7 (20.0)
8	6 (17.1)
9	4 (11.4)
10	4 (11.4)

Majority of patients scored above 6 of Gleason score.

The concentration of sVE-cadherin as measured by ELISA was significantly higher in patients with PC or BPH than in the healthy control participants (PC=19.61±5.40, BPH=18.10±4.73, Control=0.4±0.25 ng/ml, $p<0.001$). However, there was no difference between patients with either disease (Figure 1). Soluble VE-cadherin in patients with PC or BPH ranged between 10.72 and 36.94 ng/ml, and between 12.23 and 38.14 ng/ml, respectively; this value in healthy age-matched men was only between

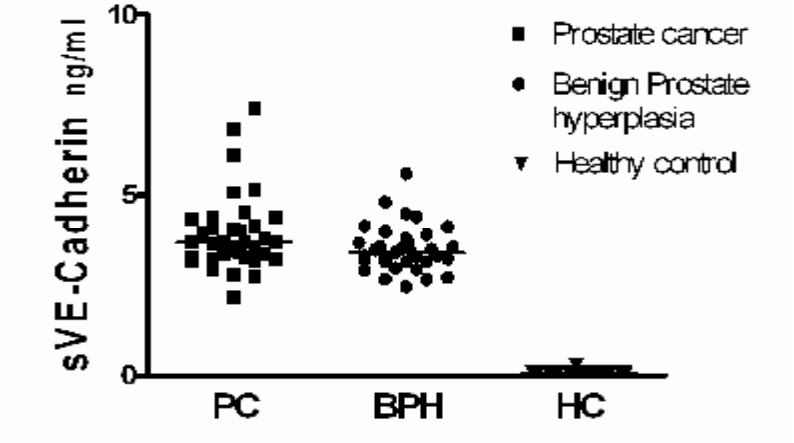

Fig. 1: Distribution of sVE-C concentration in different samples soluble vascular endothelial cadherin concentration in serum of patients with prostate cancer (PC), benign prostate hyperplasia (BPH) and healthy men were measured by ELISA. Much higher sVE-C was detected in serum of patients (PC and BPH) compared to normal individuals (p<0.0003).

0.34 and 1.39 ng/ml. In patients with PC, there was no correlation between sVE-cadherin and PSA concentration, or between any of these variables and Gleason score or age. In patients with BPH, there was no correlation between sVE-cadherin and PSA concentration, or between either of these variables and age. Similarly, no correlations between any of the variables were found in healthy individuals. However, in patients with PC whose Gleason score was higher than 8, sVE-cadherin content in the serum correlated significantly with age (p<0.0013).

Serum PSA concentration in the healthy men ranged from undetectable to 2.56 ng/ml, while in patients with PC or BPH, PSA concentrations ranged from 5.80 to 100.10 ng/ml and 3.10 to 75.20 ng/ml, respectively (Figure 2). The concentrations were significantly higher in patients with PC and BPH than in the control group (p<0.001). Also, mean PSA level in patients with PC was marginally higher than that in patients with BPH (p=0.032). In the former group, higher PSA concentrations were found in patients with higher Gleason scores (p=0.002), whereas age did not correlate with Gleason score (p=0.199).

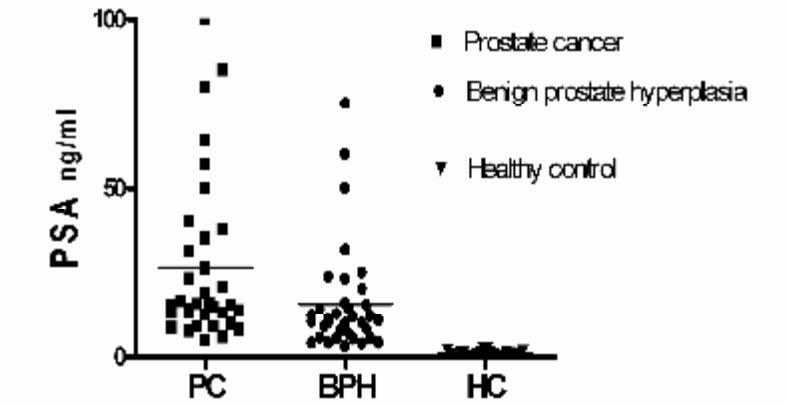

Fig. 2: Distribution of PSA concentration in different samples. Prostate specific antigen (PSA) concentration in serum of patients with PC, BPH and healthy men were measured by ELISA. Much higher PSA was detected in serum of patients groups (PC and BPH) compared to healthy individuals. The mean concentration of PSA in PC patients was slightly higher than BPH patients (p<0.03).

Discussion

The introduction of PSA as a biomarker to identify those at high risk for PC has saved the life of many patients worldwide. However, the initial cutoff values set for PSA may no longer be optimal since PSA level is neither sensitive nor specific enough for current clinical needs and yields both false positive and false negative results within "normal" ranges.[12] Moreover, it has been proved that a significant number of cancers remain undetected in patients with low PSA concentrations[13] although a significant correlation between PSA concentration and disease score was found among the PC tested patients in our study. The authors of "The Prostate Cancer Prevention Trial"[14] also concluded that there was no PSA concentration below which PC can be ruled out, and no cutoff point above which PC can be assured. Furthermore, PSA levels in men who have undergone treatment for PC are completely independent of the reference ranges.[14] Therefore, both clinicians and researchers are actively seeking to improve the accuracy of PC detection with new molecular biomarker targets.

The most useful and convenient markers are those that can be measured in blood samples, which can be analyzed more easily than tumor biopsies. Recently, Bensalah comprehensively compared a series of blood-based biomarkers for PSA, such as early prostate cancer antigens, insulin-like growth factor-I (IGF-I) and its binding proteins (IGFBP-2 and IGFBP-3), in patients with PC.[15] None of these markers managed to identify all the patients with PC among men suspected of having the disease, so further research is recommended to find one or more new targets.

Vascular endothelial cadherin is an essential adhesion molecule for angiogenesis, which is a vital process for vascularization, growth and metastasis in tumors larger than 2 mm^2.[16] Immunohistochemical and real-time quantitative PCR studies of vascular tissues have documented that VE-cadherin is a potentially informative indicator of blood vessel density in cancer tissues.[17,18] VE-cadherin can be shed from vascular cells and released to the circulation as a soluble adhesion molecule. Therefore, the increased concentration of this molecule in the serum may reflect a high degree of angiogenesis in the tumor or cadherin production by the tumor itself.[19,20] High concentrations of serum soluble VE-C have been found in patients with atherosclerosis and untreated multiple myeloma.[21,22] Similarly, increased amounts of sVE-cadherin were found in the serum of un-treated patients with colorectal cancer.[23] It has been proposed that the increase in serum sVE-cadherin may also be a protective reaction, since this factor was found to cause apoptosis and growth inhibition in a breast cancer cell line.[24] Either as cancer enhancer due to its angiogenic properties, or as an apoptosis-promoting factor, the presence of high serum concentration of sVE-cadherin may identify men at high risk for PC or BPH.

To the best of our knowledge, this is the first study showing increased concentrations of sVE-cadherin in the sera of patients with PC and BPH compared to that in the healthy men. Using ELISA assay with appropriate detection sensitivity, we were able to show significant differences between serum content of this soluble adhesion molecule in the healthy individuals and the patients. This finding is evidence of the ability of sVE-cadherin to distinguish men who are likely to have PC or BPH from men at no risk; however, it does not appear to distinguish perfectly between benign and malignant disease. In spite of the high concentration of sVE-cadherin in all the patients we tested, this figure did not correlate with the Gleason score. However, sVE-cadherin concentration did correlate with age in patients with PC whose Gleason score was 8-10, a result suggestive of an association between this marker and malignancy. Similarly, some studies have reported a link between TGF and Gleason score only in high-grade PCs, whereas others have found no such association.[25,26]

Although our findings provide evidence of a possible diagnostic role for soluble vascular endothelial cadherin in patients with PC, further prospective studies involving follow-up of large groups of patients with PC, BPH and other related diseases are needed to more fully elucidate the roles, potential usefulness and clinical importance of this marker.

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Conflict of interest: None declared.

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