Putative protein markers in the sera of men with prostatic neoplasms

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OBJECTIVE

To describe the preliminary identification of serum proteins that may be diagnostic markers in prostate cancer.

PATIENTS AND METHODS

The study included 11 men referred for treatment of localized prostate cancer, 12 with benign prostatic hyperplasia (BPH) and 12 disease-free controls. For serum protein analysis, the protein-chip array surface-enhanced laser desorption/ionization (SELDI) technique was used (Ciphergen Biosystems, Fremont, CA). SELDI combines protein-chip technology with time-of-flight mass spectrometry, and offers the advantages of speed, simplicity and sensitivity.

RESULTS

Three protein peaks were identified in the serum of men with prostate cancer and BPH, but not in controls, with relative molecular masses of 15.2, 15.9 and 17.5 kDa. These three proteins were significantly associated with BPH and prostate cancer when compared with controls ($P = 0.001$, 0.004, and 0.011, respectively, Kruskal–Wallis test). Interestingly, the 17.5 kDa protein was more abundant in five men with stage T1 prostate cancer than in eight with stage T2 ($P = 0.016$, two tailed Mann–Whitney *U*-test corrected for ties).

CONCLUSIONS

These proteins, particularly the 15.9 kDa one, may be used for the diagnosis or monitoring of prostate cancer and differentiation from BPH, and have the potential for antibody-based chip SELDI-TOF technology. Identified proteins may be targets for immunotherapy.

KEYWORDS

prostate cancer, diagnosis, prognosis, marker protein

INTRODUCTION

The PSA level, Gleason score and stage at diagnosis are currently the most reliable markers of prostate cancer prognosis and tumour aggressiveness [1]. Moreover, the widespread use of serum markers during cancer screening has led to the belief that there may be tumour markers yet to be discovered that offer better specificity and sensitivity than PSA.

Proteomics, the analysis and characterization of overall protein modifications, has added to the understanding of gene function and can aid in discovering biomarkers and/or therapeutic targets. In the past, most proteomic studies were either conducted using tumour cell lines or homogenized bulk tissue [2]. More recently, a protein biochip immunoassay has been used to assess prostate specific membrane antigen [3,4] and the serum protein (proteome) pattern in patients with prostate cancer [5]. In the present report we describe the detection of three serum proteins that may be a prognostic factor in prostate cancer.

PATIENTS AND METHODS

Suitable participants in the study were identified through urology, radiation oncology and other clinics. The accrual period was 3.5 years (1999–2002). All eligible patients were asked to participate, with the eligibility criteria being those with localized prostate cancer initially diagnosed by increasing PSA or an abnormal DRE, and with a histological confirmation of the diagnosis (11 men). All patients with BPH (12 men) were under treatment for the condition and had had their diagnosis confirmed with at least one needle biopsy of the prostate. The 12 men in the control group had no history of cancer or treatment for BPH, and were matched by age to those in the BPH and cancer group. All participants gave informed consent and the study had Institutional Review Board approval.

For serum protein analysis, the protein-chip array surface-enhanced laser desorption/ionization (SELDI) technology was used (Ciphergen Biosystems, Fremont, CA). SELDI combines protein-chip technology with time-of-flight mass spectrometry, and offers the advantages of speed, simplicity and sensitivity. Briefly, each protein-chip array has several spots that contain functional groups, chemical or biological docking sites, for the selective binding and washing of proteins/peptides from complex mixtures. After the serum sample was applied to the surface, unbound proteins and interfering substances were washed away. A solution containing laser energy-absorbing molecules, often referred to as a matrix, was then added and allowed to dry. In this fashion, the laser energy absorbing matrix molecules co-crystallizes with the adsorbed proteins. The captured proteins were then detected using laser desorption ionization time-of-flight mass spectrometry [3].

Samples were diluted (1 : 2 v/v) with 10% acetonitrile in PBS containing 50 mmol/L NaCl; 2 μL plasma aliquots were loaded onto hydrophobic H4-type SELDI chips, and dried at room temperature. The laser energy-absorbing molecule (matrix) was sinapinic acid. The mass range to 100 kDa was optimized in the 5–20 kDa range; at a laser

intensity of 270, the selected sample spot was exposed to the laser beam at 13 different positions, with five shots for every position.

RESULTS

Three protein peaks were detected that were present in the serum of men with prostate cancer and BPH, but not in the controls. The molecular masses were determined to an accuracy of 0.1–0.15%. The nominal (and mean) molecular masses in the prostate cancer patients were: 15.2 kDa (15 208 Da, SD 13.5, n = 9); 15.9 kDa (15 970 Da, SD 23.1, n = 9); 17.5 kDa (17 500 Da, SD 30.1, n = 7). The same for the BPH patients were: 15.2 kDa (15 224 Da, SD 4.28, n = 4); 15.9 kDa not detected in any BPH patient; 17.5 kDa (17 506 Da, SD 30.3, n = 4).

Figure 1 shows mass spectra (and the useful gel-type expression) of four serum samples, from two patients with prostate cancer and one each from a patient with BPH and a control. The spectrum (a) is from a patient with prostate cancer expressing the highest intensities for the putative markers. Spectrum (b) and (c) show 'average' patterns from patients with prostate cancer and BPH, respectively.

None of the potential markers were detected in the 12 controls analysed. Comparing the measured intensities (Table 1) shows that the 15.9 kDa marker appeared in nine of the 11 patients with prostate cancer but was absent in all 12 with BPH. The intensity of the 15.2 kDa marker was twice as high in prostate cancer (nine of 11) than in BPH (four of 12). The intensities of the 17.5 kDa marker were essentially the same in the BPH and cancer groups, but its frequency in cancer (seven of 11) was greater than in BPH (four of 12).

Using the Kruskal-Wallis nonparametric test, the three proteins were significantly associated with BPH and prostate cancer when compared with controls ($P = 0.001$, 0.004 and 0.013, respectively). Of potential interest is that the 17.5 kDa protein was greater in five men with stage T1 prostate cancer than in eight with stage T2 ($P = 0.016$, two-tailed Mann-Whitney *U*-test corrected for ties).

DISCUSSION

Many prostate cancers are indolent and cause no problem, but currently it is impossible to identify such tumours with certainty. With more and better biomarkers, some older men might be spared the rigours of radiation therapy and/or surgery, and their complications.

TABLE 1 The differential expression of peak intensities (arbitrary). The markers were not detected in the control samples

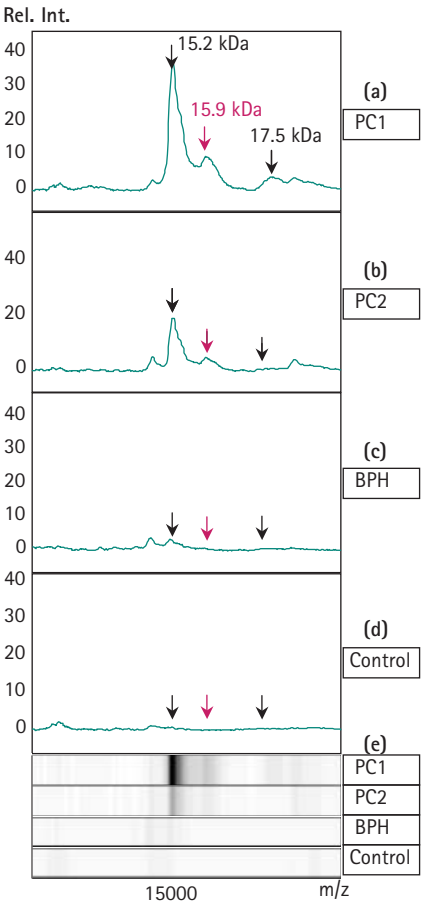
	15.2 kDa		15.9 kDa		17.5 kDa	
	Cancer	BPH	Cancer	BPH	Cancer	BPH
No. positives/total patients	9/11	4/12	9/11	0/12	7/11	4/12
Mean	12.0	5.8	3.1	–	2.8	2.6
SD	10.5	0.2	2.8	–	0.9	1.2
Range	4.5–36.9	5.6–6.1	1.1–9.7	–	1.9–4.0	1.8–4.0

Because none of these potential markers appeared in prostate disease-free controls, the proteins detected in this study might be such biomarkers for the diagnosis and monitoring of prostate cancer. The 15.9 kDa protein, which was present in 9/11 cancer patients but not in 12 BPH patients, may be used for differential diagnosis. After further confirmation of the present findings, it might be worthwhile to isolate and identify these proteins for the development of highly specific antibody-based chip SELDI-TOF methodologies and, eventually, as targets for immunotherapy.

The difficulty of reproducible measurements is a well-known limitation of the present systems of SELDI-time-of-flight mass spectrometry. One obvious approach to obtain more reproducible measures is to use internal standards, e.g. insulin. We established in a previous study [6] that the molecular mass of insulin can be determined accurately and reproducibly by the present methods (24 samples, mean 5736, SD 4.2, range 5726–5756 Da).

The identities of these proteins are under investigation by mass spectrometric analysis using techniques similar to the approach used to identify the putative 8.9 kDa marker found in colon cancer, i.e. peptides obtained using digestion with proteases are analysed by electrospray mass spectrometry to obtain both MS and MS/MS spectra, followed by comparison of the identified peptides with data available in protein databases [7].

FIG. 1. Protein profiles, (a) to (d) mass spectral format, (e) gel format. PC = prostate cancer; (a) = PC sample in which markers expressed at highest intensity; (b) = PC sample in which markers are expressed at average intensity; (c) = benign prostatic hyperplasia = BPH. (d) = control, subjects without prostate disease. Arrows show putative markers never before reported for prostate diseases.



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Abbreviations: **SELDI**, surface-enhanced laser desorption/ionization.