ORIGINAL ARTICLE – CANCER RESEARCH

Further evaluation of uPA and PAI-1 as biomarkers for prostatic diseases

John Akudugu · Antonio Serafin · Lothar Böhm

Abstract

Purpose To assay for uPA and PAI-1 in prostate tissue from 40 patients with prostatic disease and to examine the robustness of the correlation of the uPA/PAI-1 ratio with benign prostatic hyperplasia (BPH) and prostate cancer (PCa), previously identified in a different cohort of 62 patients.

Methods uPA and PAI-1 were extracted from liquid N_2 frozen homogenised prostate tissue with TRIS/Triton pH 8.5 buffer and measured by ELISA (FEMTELLE).

Results The concentration of uPA (mean ± SD) was found to be 0.1177 ± 0.0266 (range 0.0070–0.7200; $n = 30$) and 0.1092 ± 0.0130 (range 0.0040–0.7800; $n = 70$) for PCa and BPH patients, respectively. The concentration of PAI-1 was found to be 5.236 ± 0.688 ng/mg protein (range 1.10–15.19; $n = 30$) and 4.975 ± 0.501 ng/mg protein (range 0.20–25.00; $n = 70$) for PCa and BPH patients, respectively. The mean uPA/PAI-1 ratio was found to be 0.0479 ± 0.0060 (range 0.0043–0.1200; $n = 30$) in PCa samples and was significantly higher than BPH samples where the ratio was 0.0332 ± 0.0023 (range 0.0040–0.0860; $n = 70$) ($P = 0.0064$). In PCa patients older than 68 years, the uPA/PAI-1 ratio was above 0.050 reaching 0.100 in 73-year-old patients.

Conclusions Evaluation of 100 patients with prostatic pathologies (70 PCa; 30 BPH) shows the uPA/PAI-1 ratios in PCa patients to be significantly higher than in BPH patients. This is fully consistent with a previous study on 62 patients (16 were PCa; 46 BPH) where the ratios were 0.055 and 0.031 for PCa and BPH patients, respectively ($P = 0.0028$). In older PCa patients, uPA/PAI-1 ratios tend to be higher.

Keywords Prostate tissue resections · uPA/PAI-1 markers · Correlation with prostatic disease

Introduction

Urokinase plasminogen activator (uPA) and its inhibitor PAI-1 have been implicated in various processes of tissue differentiation and metastasis (Dano et al. 1985; Andreasen et al. 1997; Egeblad and Werb 2002), and elevated uPA and uPAR levels have been found to be associated with features of aggressive prostate cancer (PCa) (Shariat et al. 2007a, b). No definitive role of the involvement of uPA and PAI-1 in PCa has yet emerged. It also remains uncertain whether either of these two components predominates in different stages of disease to set a distinguishing feature which could be used for diagnosis or prognosis.

In a previous paper, we have come to the conclusion that the uPA/PAI-1 ratio in tissue resections from 16 patients with PCa and 46 patients with benign prostatic hyperplasia (BPH) is statistically different (Böhm et al. 2013). This finding was underpinned by the observation that the dependence of the PAI-1 concentration on the uPA content in BPH samples was very steep, while it was very shallow for PCa samples (Böhm et al. 2013). In the following, we present additional data from 14 patients with PCa and 24 with BPH to further examine the robustness of the uPA/PAI-1 ratio as an indicator of prostate cancer.

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Table 1 Patient data and diagnosis

Patient	Age	PSA ng/ml	uPA ng/mg protein	PAI-1 ng/mg protein	uPA/PAI-1	TNM status	Gleason	Diagnosis
1	61	16.39	0.13	1.78	0.0730			BPH
2	66	4.56	0.16	3.93	0.0407			BPH
3	77	5.06	0.06	1.92	0.0313			BPH
4	65	0.76	0.12	3.88	0.0309			BPH
5	71	9.00	0.10	1.70	0.0588			BPH
6	72	1.00	0.13	2.46	0.0528			BPH
7	82	1.40	0.04	4.05	0.0099			BPH
8	62	1.00	0.05	2.54	0.0197			BPH
9	74	5.28	0.14	3.19	0.0439			BPH
10	56	3.01	0.08	3.51	0.0210			BPH
11	65	2.27	0.19	3.86	0.0492			BPH
12	62	1.86	0.07	1.60	0.0438			BPH
13	69	0.45	0.17	2.33	0.0729			BPH
14	67	1.44	0.17	2.66	0.0639			BPH
15	61	1.19	0.04	2.38	0.0168			BPH
16	73	26.00	0.26	11.42	0.0228	T2NxMx		BPH
17	52	3.49	0.01	2.51	0.0040			BPH
18	68	12.36	0.11	2.42	0.0455			BPH
19	70	29.45	0.21	7.89	0.0266			BPH
20	68	14.68	0.17	9.98	0.0170			BPH
21	72	5.29	0.16	4.12	0.0388			BPH
22	60	0.45	0.03	1.79	0.0168			BPH
23	73	1.20	0.08	2.51	0.0319			BPH
24	70	0.68	0.13	2.15	0.0605			BPH
Range		**0.45–29.45**	**0.01–0.26**	**1.60–11.42**	**0.004–0.073**			
25	71	37.73	0.05	1.38	0.0362	T4NxMx	9	PCa
26	53	137.00	0.36	7.39	0.0487	T4NxMx	9	PCa
27	75	2.63	0.16	2.95	0.0542			PCa
28	64	1509.80	0.10	6.59	0.0152	T4NxM1		PCa
29	75	2.18	0.17	1.84	0.0924		6	PCa
30	76	2.80	0.08	1.79	0.0447	T4NxMx	9	PCa
31	63	423.99	0.25	5.46	0.0458	T3NxMx	8	PCa
32	67	20.43	0.12	3.91	0.0307	T4NxMx	10	PCa
33	62	2.27	0.07	1.56	0.0449	T1b	6	PCa
34	69	31.72	0.09	5.33	0.0169	T2NxMx	6	PCa
35	58	36.20	0.18	4.47	0.0403	T2NxM1	7	PCa
36	88	20.80	0.38	8.86	0.0429	T3NxMx	8	PCa
37	67	19.70	0.64	15.19	0.0421	T2NxMx	9	PCa
38	84	3,400.00	0.08	11.47	0.0070		9	PCa
Range		**2.18–3,400.0**	**0.05–0.64**	**1.38–15.19**	**0.007–0.092**			

See scoring system based on PSA, DRE and histopathology as described in Table 1 "Materials and methods"

Materials and methods

The additional patient cohort consisted of 38 patients from the Tygerberg Academic Hospital in Parow and the Gatesville Medi Clinic in Athlone, both in Cape Town (South Africa), of which 24 patients were found to be BPH and 14 were PCa (Table 1). Identification of disease stage was by a special scoring system based on histopathology, PSA, Gleason Index and DRE, as described previously (Böhm et al. 2013). Expression of uPA and PAI-1 was determined in prostate tissues from this cohort, as previously described (Jänicke et al. 1994; Vetter et al. 2007). The data were then
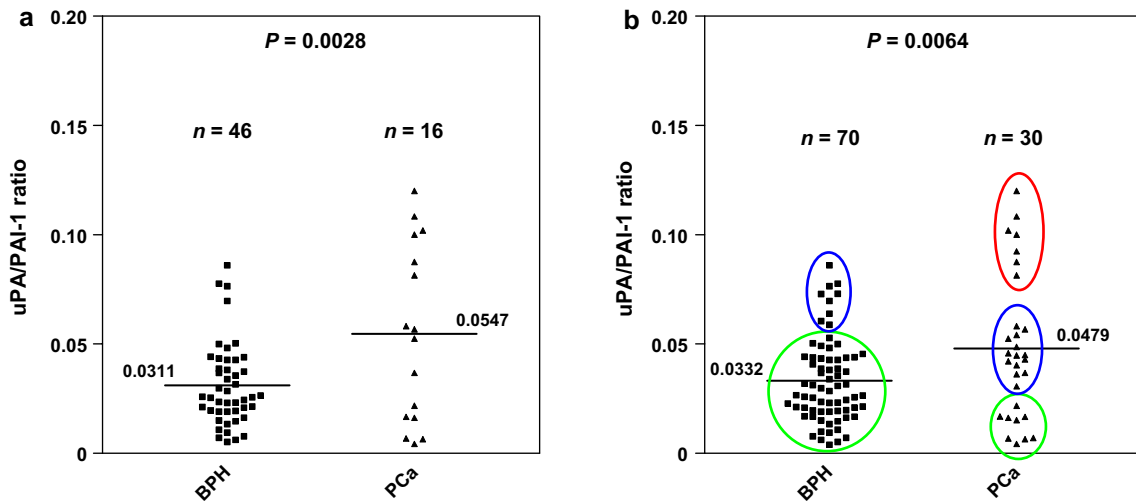
Fig. 1 Comparison of uPA/PAI-1 ratios in BPH and PCa tissues: **a** from 62 patients (Böhm et al. 2013) and **b** from 100 patients (Böhm et al. 2013 and Table 1). *Horizontal lines* represent the mean uPA/PAI-1 ratio in each group of patients

combined with those published in a preceding study (Böhm et al. 2013), and analysed, with respect to patient age and marker content. A total of 100 patient samples were included in this analysis.

Results and discussion

Using the data from the contingent of 62 patients presented previously (Böhm et al. 2013), we show that uPA/PAI-1 ratios range from 0.0053 to 0.0860 in the BPH group, while the ratios in the PCa group range from 0.0042 to 0.1200 (Table 1 and Fig. 1a). The corresponding means were found to be 0.06 and 0.03 for the PCa and BPH groups, respectively (Fig. 1b). Addition of 40 new samples and analysis of the resultant 100 patient contingent showed the uPA/PAI-1 ratios in the BPH group to be 0.0332 ± 0.0023 and in the PCa group to be 0.0479 ± 0.0060 (Fig. 1b). In the enlarged 100 patient contingent, the uPA/PAI-1 ratios for PCa patients are again higher than for BPH patients, the difference between patient groups being significant at the level of $P = 0.0064$.

Figure 2 shows that in BPH patients, the PAI-1 content is correlated positively with uPA content, each elevated uPA content corresponding to a high PAI-1 content. The correlation coefficient emerged as 0.54 ($P = 0.0001$). In PCa patients, on the other hand, the influence of uPA on PAI-1 content is very small, showing a very low dependence upon uPA content ($r = 0.37$, $P = 0.047$). The marked differences in PAI-1 expression between the two patient groups are consistent with the large variation of uPA/PAI-1 ratios in PCa patients and the much smaller variation in BPH patients (Fig. 1b).
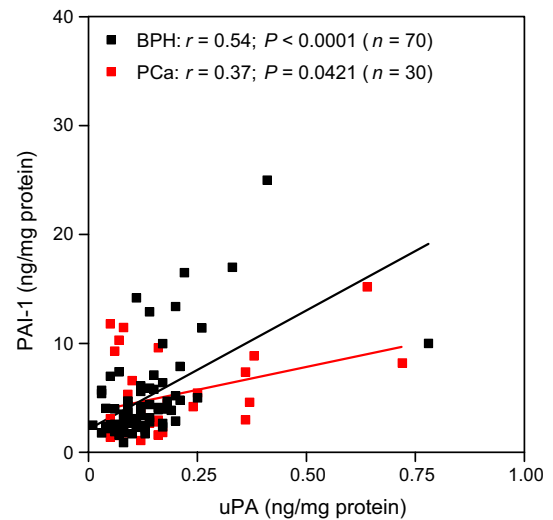
Fig. 2 Dot plot of PAI-1 concentration as a function of uPA concentration in BPH and PCa patient samples

Interestingly, the results in Fig. 1b show distinct clusters of patients on the basis of the uPA/PAI-1 ratio. Two and three subgroups are indicated for the BPH and PCa groups, respectively. When patient age was entered as a variable, another difference between groups emerged. In the BPH subgroups, our patients were nearly identical in age, with average ages of 67.69 years ($n = 61$) and 67.67 years ($n = 9$), respectively. In these subgroups, the uPA/PAI-1 ratios ranged between 0.025 and 0.075 showing a threefold variation (Fig. 3). In the PCa group, on the other hand, nine patients with an average age of 67.11 years show a ratio as low as 0.012 increasing to 0.045 for 14 patients of average age of 67.14 years and further to an average ratio of
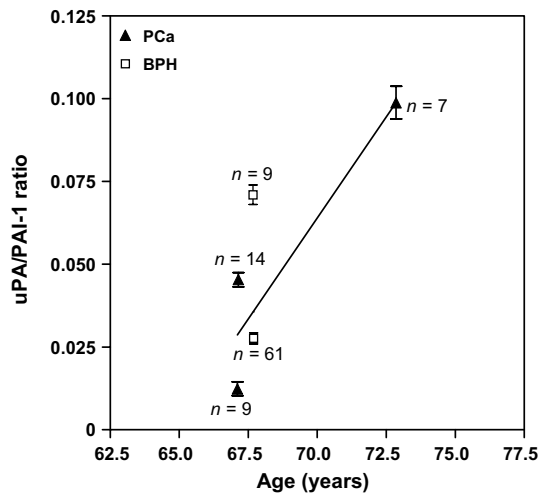
Fig. 3 Correlation between patient age and uPA/PAI-1 ratio in BPH and PCa tissue samples

0.099 for seven patients averaging 72.86 years. The indication that uPA/PAI-1 ratios above 0.05 are only seen in patients above the age of 67.5 years implies that the utilisation of the uPA/PAI-1 ratio for diagnostic purposes must deal with the age factor and cannot be straightforward. Higher ratios have already been shown to predominate in PCa patients (Fig. 1b) and have been suggested as a promising marker candidate for malignancy (Böhm et al. 2013). A cautious interpretation of the present data on marker abundance (Fig. 1), marker interrelations (Fig. 2) and age (Fig. 3) would be that ratios >0.05 in patients near and above the age of 67 years arise from prostate cancer, whereas ratios <0.05 and an age below 67 years arise from a benign pathology. The evaluation of a greater cohort of patients must deal with our finding that prostate abnormalities occur in a fairly narrow age group. Age-related changes of fibrolytic factors and cardiovascular ageing in the elderly have been postulated to be associated with PAI-1 induction (Yamamoto et al. 2005). Recruitment of larger sample numbers from BPH patients across different age groups would be needed to fully elucidate the separate influence of age on marker expression.

Our present conclusion is that the uPA/PAI-1 ratio is not a coincidental numerical phenomenon. This is supported by three observations: firstly, that the ratios for PCa pathologies are significantly higher than for BPH pathologies (Fig. 1). Secondly, that the ratio is strongly dependent upon the uPA concentration in BPH pathologies but not in PCa pathologies (Fig. 2). Thirdly, that the ratio for the PCa but not for BPH cases shows a dependence upon patient age (Fig. 3). Examination of prostate resections by immunocytochemistry has led to the conclusion that overexpression of one marker is not more likely to be

associated with overexpression of the other marker (Gupta et al. 2009). This is fully consistent with our finding of PAI-1 expression being nearly independent of uPA expression in PCa (Fig. 2), while PAI-1 expression in BPH correlates with uPA concentration (Fig. 2). Work on thrombotic disorders has drawn attention to the fact that PAI-1, elevated in the elderly, can also be observed in a variety of pathologies associated with ageing, e.g. obesity, insulin resistance and vascular sclerosis (Yamamoto, 2005). However, age-related diseases other than prostatic abnormalities were not manifest in our patient cohort. Higher uPA/PAI-1 ratios (Fig. 3) could arise from a reduction of PAI-1 concentration or an increase in the uPA concentration. The uPA concentration is shown to be more than one order of magnitude lower than the PAI-1 concentration and ranges from 0.01 to 0.64 ng/mg protein, while PAI-1 ranges from 1.38 to 15.19 ng/mg protein (Table 1; Fig. 1). In BPH and PCa patients, the uPA concentration is shown to range from 0.01 to 0.26 ng/mg protein and from 0.05 to 0.64 ng/mg protein, respectively, while the PAI-1 concentrations range from 1.60 to 11.42 ng/mg protein and 1.38–15.19 ng/mg protein for BPH and PCa pathologies, respectively. In prostate tissues, the ranges of the two markers clearly are rather similar between pathologies. A linear approach using appropriate cut-off points for the identification of aggressive and indolent pathologies which is well established for the evaluation of early mamma carcinoma (Thomssen et al. 2009; Harbeck et al. 2013; Vetter et al. 2007) thus cannot apply here. The only common feature between prostate and mamma abnormalities is that the PAI-1 concentration in prostate tissues tends to be 20–40 times higher than the uPA concentration (Böhm et al. 2013). The results presented here on 38 patients are in agreement with our previous study on 62 patients (Böhm et al. 2013) showing that the uPA/PAI-1 ratio separates benign and malignant prostate pathologies and emerges as a candidate marker for BPH and PCa disease. The extensive research over the last 15 years on lumpectomies in early mamma carcinomas has settled on a linear model with cut-off points of 3 ng/mg protein (uPA) and 14 ng/mg protein (PAI-1) to clinically characterise aggressive and indolent tumour types (Vetter et al. 2007). As far as prostate is concerned, it is clear that additional measurements in larger cohorts of patients, and between laboratories, are needed to further evaluate the performance of these markers. We have recently demonstrated that freeze-drying of deep frozen prostate tissue homogenate extends the shelf life to well over 2 years with a minimal (10–11 %) loss of uPA and PAI-1 concentrations (Serafin et al. 2014). Exchange of the freeze-dried tissue powder from well-characterised pathologies and intercomparisons between laboratories could help to ascertain marker margins as a first step for clinical applications.

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