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Plasma Levels of Transforming Growth Factor-1β and α2-Macroglobulin Before and After Radical Prostatectomy: Association to Clinicopathological Parameters

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BACKGROUND. To study the levels of transforming growth factor-1β (TGF-β1) and of α2-macroglobulin (α2-M), a high affinity binding protein of TGF-β1, in comparison to prostate-specific antigen (PSA) in prostate cancer (PCa) patients before and up to 12 months after prostatectomy, and to correlate the results with clinicopathological parameters.
METHODS. Eighty-one patients who underwent radical prostatectomy for PCa were included in this study. Pre- and postoperatively, plasma levels of TGF-β1, α2-M and PSA were measured in the same samples by ELISA, and were correlated with pathological parameters and clinical outcomes.
RESULTS. The preoperative TGF-β1 levels were significantly elevated as compared to the controls; they showed a positive correlation with the Gleason score. Patients with initial androgen-deprivation therapy had lower TGF-β1 levels than untreated patients. Elevated concentrations of TGF-β1 levelled off 12 months after prostatectomy approaching values of healthy individuals. Decreased plasma levels of total and transformed α2-M (proteinase-complexed form) were observed in PCa. Preoperative levels of TGF-β1 but not of α2-M seem to be influenced by the body mass index (BMI).
CONCLUSIONS. Elevated TGF-β1 and decreased α2-M were consistently found in patients with PCa, and may be considered as risk factors for tumor development and progression. In comparison to PSA, the TGF-β1 levels displayed a slow decline after radical prostatectomy; this indicates that TGF-β1 is mainly produced outside the prostatic tissue. Since TGF-β1 levels are influenced by the BMI, this indicates that PCa might be sensitive to diet. *Prostate 61: 201–208, 2004.* © 2004 Wiley-Liss, Inc.

KEY WORDS: **prostate; prostate cancer; transforming growth factor-1β; prostate-specific antigen; α2-macroglobulin; body mass index; prostatectomy**

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INTRODUCTION

Prostate cancer (PCa) is one of the most common malignancies in men in the industrial states. The mortality, which is associated with PCa, is probably due to the prevalence of metastases at the time of diagnosis [1]. Although many patients are potentially cured by radical prostatectomy, more than 20–30% of men have relapse with elevated prostate-specific antigen (PSA) levels by 10 years after surgery. Androgen-deprivation therapy reduces the clinical symptoms in patients with advanced PCa. Unfortunately, however, most tumors relapse within 2 years to an androgen-independent stage. Efforts to stop the progress of PCa have been frustrated by the lack of a highly sensitive and specific screening tool to detect cancer at an early stage when it is confined within the gland. Several risk markers for PCa have been discussed, but neither of them allows the prediction of cases with imminent metastasis formation that require active management.

Cancer development is under the control of endocrine and paracrine mechanisms. The importance of these factors in prostate carcinogenesis, and, thus, of an elucidation of their features and roles, can not be overstated. The most important growth factors, presently known to regulate prostate growth, are members of the transforming growth factor-β (TGF-β) family. Normally, TGF-β1 inhibits prostate epithelial cell growth and maintains normal prostate homeostasis [2]. Paradoxically, high levels of TGF-β1 are often found in PCa tissue. Here, TGF-β1 may induce a stromal reaction promoting tumor growth and angiogenesis.

The proteinase inhibitor α2-macroglobulin (α2-M) is known to modulate the levels of TGF-β1 as well as of PSA by forming complexes, which are rapidly cleared via the cellular receptor LRP1 [3–5].

α2-M is the only inhibitor which can react with almost all proteinases of all classes. This interaction leads to inhibition of proteinases and, independent of the type of enzyme, creates a common structural change in the inhibitor molecule which then mediates binding of growth factors and cytokines and the clearance of the complexes. Changes in the level of that fraction of the inhibitor which is in complex with proteinases, commonly called transformed α2-M, may thus reflect differences of the proteolytic charge in blood and/or tissue.

This suggests that changes in the concentration of α2-M may have an impact on the activity and bioavailability of growth factors and PSA in human plasma and tissue.

Thus, the present study was aimed at examining the association of TGF-β1, α2-M, and PSA with other clinicopathological parameters in PCa.

MATERIALS AND METHODS

Patients

A total of 81 patients who underwent radical prostatectomy were evaluated in this study. The mean patient age was 62.9 years with a range of 50–77 years.

Thirty-eight patients were classified as T1-2N0M0 (group 1), 39 patients as T3-4N0M0 (group 2), and 4 patients as TxN1M0, TxN0M1, and TxN1M1 (group 3). Tumor grade was assessed by a pathologist and graded according to the WHO classification. Fifty-seven patients obtained a preoperative androgen-deprivation therapy with LH-RH analog. Patients were further subdivided according to their Gleason score and evaluated as grade 2–6 versus 7–10. Patients were grouped according to NCI/NHLBI-defined body size categories of obese (body mass index (BMI) ≥ 30 kg/m^2), overweight ($25 \leq$ BMI < 30 kg/m^2), and normal weight (BMI < 25 kg/m^2).

The control group I consisted of 28 healthy individuals, its mean age was 59.3 years (range, 51–81 years) and was used for TGF-β1 determination. Another control group (group II) composed of 43 healthy male individual with the mean age of 58.1 years (range, 50–78 years) was used for comparison of the α2-M levels.

Both control groups had no prior history of cancer or chronic disease, and displayed normal digital-rectal examination results and PSA levels less than 2.0 ng/ml.

Blood Specimens

Blood was collected from patients 2 days before surgery (−2d), 3 months (+3m), and 6–12 months (+12m) after the operation each following an overnight fasting. The blood was collected in sterile containers containing citrate and centrifuged for 20 min at 4°C at 2,000*g*. Plasma was decanted and centrifuged again at 13,000*g* for 15 min. Unless used immediately, the plasma was frozen at −80°C.

For quantitative measurements of TGF-β1 levels, the Quantikine TGF-β1 test kit (R&D Systems, Minneapolis, MN) was used. The sensitivity of the test was 7 pg/ml; the intra- and inter-assay coefficient of variation was below 12.8%.

The total concentration of α2-M in plasma was determined by means of the MacroNat®-ELISA test kit from BioMac (Leipzig, Germany). The proteinase-complexed form, or transformed α2-M, was analyzed using the MacroTrans®-ELISA test kit (BioMac). The sensitivities of the two tests were 2 ng/ml and the intra- and inter-assay coefficients of variation were below 9.6% and 9.1%, respectively.

Total PSA was immunologically assayed using the ECLIA-kit of Roche (Mannheim, Germany). The sensitivity of the test was 0.002 ng/ml; the intra- and

inter-assay coefficient of variation was below 3.8%. Every sample was run in duplicate.

This study was approved by the local Ethical Committee.

Pathological Examination

All prostatectomy specimens were examined routinely by the Department of Pathology of the St. Georg Hospital, Leipzig, Germany.

Statistical Analysis

The data were analyzed by the statistics package SPSS program for WINDOWS®. Normal distribution was tested with the Kolmogorov–Smirnov test. The levels of α2-M (Gaussian distribution) were analyzed by parametric tests (*t*-test) and non-parametric tests (Mann–Whitney U-test and Wilcoxon test) were used for analysis of PSA and TGF-β1 (non-Gaussian distribution). Association among α2-M, PSA, TGF-β1 levels, and BMI were compared by Spearman rank correlation. *P*-values less than 0.05 were considered as statistically significant.

RESULTS

Pre- and Postoperative TGF-β1 Levels

The median TGF-β1 levels of the 81 patients with PCa (−2d values) were significantly higher [12.23 (6.25, 15.28) ng/ml] than those of the 28 healthy patients (control group I) [9.47 (6.21, 12.41) ng/ml] ($P < 0.05$). The postoperative follow-up analysis revealed that the TGF-β1 levels significantly increased to 16.81 (9.78, 20.22) ng/ml at 3 months after surgery ($P < 0.05$; +3m vs. −2d). Twelve months after prostatectomy, the concentration of TGF-β1 levelled off to 9.43 (7.02, 12.64) ng/ml which was below the preoperative value and very close to the normal TGF-β1 levels of control group I (Table I). The preoperative plasma levels of TGF-β1 in patients with metastatic PCa (group 3) were much higher than those in the other cohorts. However, our sample number is limited to draw conclusions regarding the significance of that difference. In tumors with high Gleason scores [7–10], the preoperative TGF-β1 levels were significantly different from the controls [16.91 (6.57, 19.02) ng/ml vs. 9.47 (6.21, 12.41) ng/ml] ($P < 0.05$), whereas no difference was found between low Gleason score tumors and controls. Preoperative TGFβ1-levels were positively associated with Gleason scores ($r = 0.38$; $P < 0.05$). This indicates that high preoperative TGF-β1 levels may predict a high tumor aggressiveness. Furthermore, we found that androgen-deprived patients revealed lower TGF-β1 levels than the non-treated patients ($P > 0.05$). These differences disappeared after prostatectomy and cessation of therapy (−2d vs. +12m).

Among 81 patients, no correlation between preoperative TGF-β1 and PSA was found ($r = 0.09$, $P > 0.05$) (Tables I and II).

When the patients were grouped according to their BMI, different median levels of TGF-β1 were found both pre- (−2d) and postoperative (+3m) (Fig. 1). Apparently, the preoperative levels of TGF-β1 increased significantly with body weight [8.23 (5.46, 12.43) ($BMI < 25\ kg/m^2$) vs. 14.51 (8.53, 18.07) ($BMI > 30\ kg/m^2$); $P = 0.018$]. After operation, the differences between the BMI groups disappeared and the values approached to TGF-β1 levels of the control group. No significant deviation could be observed in the lean patient group over the time of observation. These data suggest that overweight might be a risk factor for TGF-β1 overproduction.

TABLE I. Pre- and Postoperative Characteristics of TGF-β1 Plasma Levels (ng/ml) [Median (25th, 75th Centile)] in 81 Patients Undergoing Radical Prostatectomy

	Preoperative (−2d)	3 months postoperative (+3m)	6–12 months postoperative (+12m)
Prostectomy patients (n = 81)	12.23 (6.25, 15.28)	16.81 (9.78, 20.22)	9.43 (7.02, 12.64)
T1-2N0M0 (n = 38)	13.21 (6.84, 15.26)	16.80 (8.74, 21.70)	9.91 (7.06, 12.85)
T3-4N0M0 (n = 39)	13.95 (7.79, 17.85)	16.91 (10.75, 22.51)	9.44 (6.89, 12.67)
TxN1M0, TxN0M1, TxN1M1 (n = 4)	20.20 (9.82, 25.20)	27.24 (10.21, 33.44)	9.97 (7.39, 12.15)
Androgen deprivation			
Yes (n = 57)	10.00 (6.02, 15.13)	15.09 (8.51, 21.03)	9.09 (6.86, 12.97)
No (n = 24)	14.14 (12.06, 20.53)	17.54 (10.11, 22.06)	9.94 (7.43, 12.44)
Gleason score			
2–6 (n = 46)	10.56 (6.43, 15.93)	14.54 (8.42, 21.87)	9.02 (6.61, 12.78)
7–10 (n = 35)	16.91 (6.57, 19.02)	17.80 (8.81, 22.11)	9.57 (7.37, 12.34)

d, day; m, month.

TABLE II. Pre- and Postoperative Characteristics of PSA Plasma Levels (ng/ml) [Median (25th, 75th Centile)] in 81 Patients Undergoing Radical Prostatectomy

	Preoperative (−2d)	3 months postoperative (+3m)	6–12 months postoperative (+12m)
Prostectomy patients (n = 81)	6.53 (1.27, 10.12)	0.41 (0.03, 0.62)	0.35 (0.03, 0.61)
T1-2N0M0 (n = 38)	4.91 (0.91, 8.34)	0.22 (0.04, 0.41)	0.17 (0.03, 0.32)
T3-4N0M0 (n = 39)	7.11 (1.71, 12.59)	0.59 (0.12, 2.21)	0.49 (0.03, 1.39)
TxN1M0, TxN0M1, TxN1M1 (n = 4)	26.14 (4.31, 80.1)	0.73 (0.31, 1.13)	0.62 (0.43, 2.1)
Androgen deprivation			
Yes (n = 57)	4.01 (0.84, 7.37)	0.21 (0.05, 2.61)	0.19 (0.04, 1.21)
No (n = 24)	13.15 (6.26, 17.02)	0.12 (0.02, 0.96)	0.09 (0.03, 0.13)
Gleason score			
2–6 (n = 46)	4.89 (0.98, 8.20)	0.08 (0.01, 0.47)	0.11 (0.01, 0.92)
7–10 (n = 35)	6.15 (1.39, 10.91)	0.89 (0.01, 0.47)	0.71 (0.02, 1.21)

d, day; m, month.

Pre- and Postoperative PSA Levels

The median preoperative PSA level of the 81 prostate carcinoma patients was 6.53 (1.27, 10.12) ng/ml (Table II). As expected, the PSA concentration significantly declined shortly after prostatectomy, and 6–12 months after surgery, only small amounts were measured. We found higher PSA levels in more advanced tumor. Looking at the preoperative PSA values in the hormone-treated and -untreated groups, we corroborated a reduced PSA production in the prostate as the well-known effect of androgen deprivation. In addition, we found higher preoperative PSA values in patients with high Gleason scores if compared to low Gleason score-tumors. However, neither of these differences was statistically significant ($P > 0.05$).

Pre- and Postoperative α2-M Levels

As clearly shown in Figure 2, the plasma levels of α2-M were significantly decreased in all PCa patients if compared to the control group (1.34 ± 0.45 mg/ml vs. 1.75 ± 0.65 mg/ml; $P < 0.01$). After prostatectomy, the levels of α2-M increased non-significantly at +3m

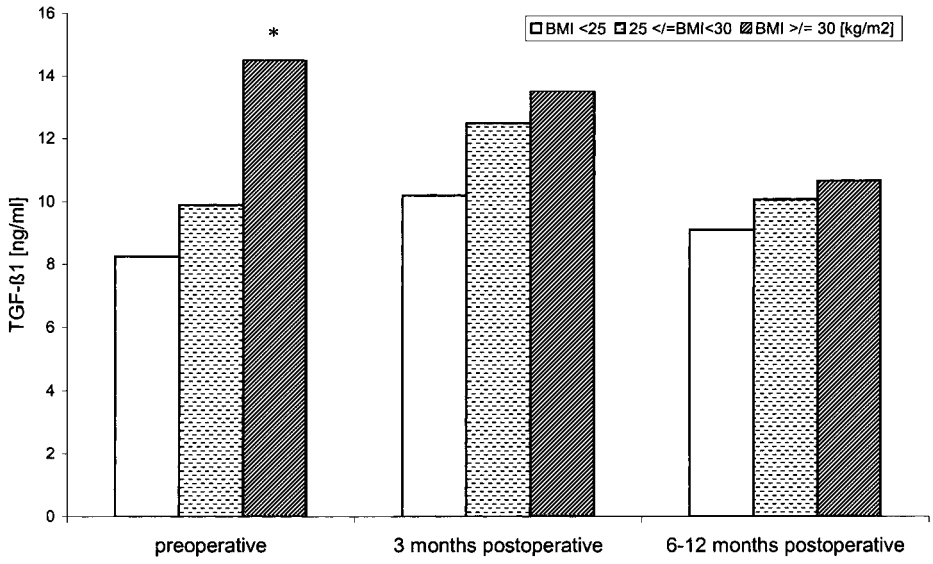

Fig. 1. Pre- and postoperative characteristics of median TGF-β1 levels (ng/ml) in 81 PCa patients in dependence of the body mass index. Eighty-one patients were grouped according to their body mass index (BMI) shown by the inset. The concentrations of TGF-β1 expressed as ng/ml (ordinate) was measured in the plasma obtained preoperative [8.23 (5.46, 12.43), 9.87 (6.11, 14.10), and 14.51 (8.53, 18.07)] for the different BMI groups, respectively) and postoperative at 3 months [10.20 (6.33, 13.41), 12.56 (7.12, 16.47), and 13.52 (6.47, 16.38)] for the different BMI groups, respectively) and 6 – 12 months [9.11 (7.31, 11.32), 10.07 (8.02, 12.40), and 10.62 (7.93, 14.69)] for the different BMI groups, respectively) after prostatectomy. Data presentation: median (25th, 75th centile).*$P < 0.05$.

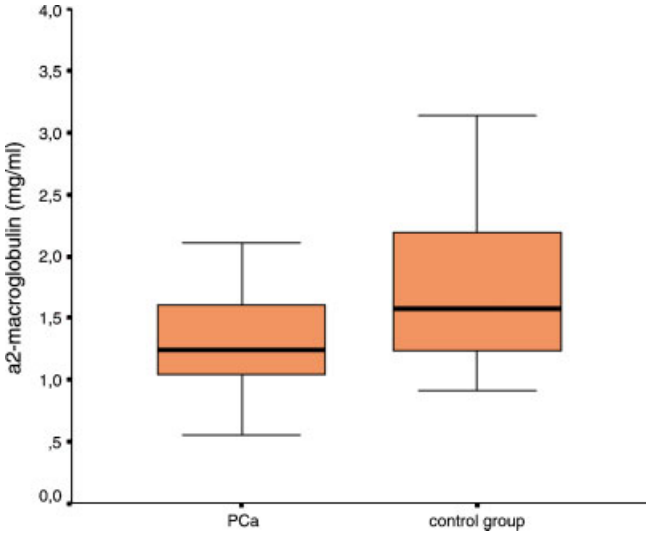

Fig. 2. Levels of α2-macroglobulin in healthy male individuals and PCa patients. The concentration of total α2-M was determined by ELISA in healthy individuals (control group II) and in 81 patients suffering from PCa (−2d values). Box boundaries denote 25th and 75th centiles. The median is the solid line in the boxes. The mean α2-M levels were 1.75 ± 0.65 mg/ml and 1.34 ± 0.45 mg/ml, respectively ($P < 0.01$). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

and finally dropped to 1.28 ± 0.41 mg/ml at 6–12 months (+12m) after surgery (+3m vs. +12m; $P < 0.05$) (Table III). Noteworthy, there were no overall differences between the α2-M levels before and 6–12 months after prostatectomy and the removal of the prostate gland did not cause any elevation of the α2-M levels towards the values of the control group.

As shown in Table III, the levels of α2-M were higher in metastatic PCa than in the other cohorts, irrespective of the time of evaluation (−2d, +3m, and +12m). Again, due to the low case number (n = 4), these changes were not significant ($P > 0.05$). No significant effect of androgen-deprivation therapy or of Gleason score, on the α2-M levels was verifiable.

As tumor growth and invasion are accompanied by the activation of proteolytic enzymes such as metalloproteinases or kallikreins like PSA, it was to be expected that enzyme inhibition by α2-M will be reflected by an increased fraction of transformed α2-M (i.e., a proteinase-complexed form) in plasma. As demonstrated in Table IV, the levels of transformed α2-M in PCa patients at the preoperative stage amounted to 2.80 ± 1.40 μg/ml, which was significantly lower compared to healthy controls (5.3 ± 4.31 μg/ml; $P < 0.01$). This initial decline was followed by an continuous increase at +3m. This is of particular interest because this fraction of α2-M reflects the proteolytic charge of the blood. We found a correlation between total α2-M and transformed α2-M at different times of blood analysis (−2d: r = 0.40, $P < 0.01$; +3m: r = 0.33, $P < 0.01$; +6–12m: r = 0.44, $P < 0.01$). Further analysis of a possible association between transformed α2-M levels, Gleason score, and androgen deprivation, or tumor stage revealed no significant association between those parameters.

Since α2-M is an important inhibitor of PSA, we searched for an association between transformed α2-M and plasma PSA at different stages of the disease. It was expected that higher PSA levels would trigger higher concentrations of transformed α2-M. However, no such correlation was found (r = 0.06; $P = 0.54$).

When we finally analyzed the fraction of transformed α2-M, expressed as a ratio of transformed α2-M to total α2-M, a significantly decreased ratio was found in preoperative patients as compared to the controls (group II) (0.31 ± 0.26; $P < 0.01$) (Fig. 3). This may reflect a lack of proteolytic activity in plasma. Twelve months after prostatectomy, the ratio was restored.

TABLE III. Pre- and Postoperative Characteristics of Total α2-M Plasma Levels (mg/ml) in 81 Patients Undergoing Radical Prostatectomy

	Preoperative (−2d)	3 months postoperative (+3m)	6–12 months postoperative (+12m)
Prostatectomy patients (n = 81)	1.34 ± 0.45	1.39 ± 0.49	1.28 ± 0.41
T1-2N0M0 (n = 38)	1.34 ± 0.44	1.36 ± 0.30	1.23 ± 0.32
T3-4N0M0 (n = 39)	1.34 ± 0.48	1.40 ± 0.60	1.29 ± 0.49
TxN1M0, TxN0M1, TxN1M1 (n = 4)	1.37 ± 0.22	1.63 ± 0.79	1.57 ± 0.30
Androgen deprivation			
Yes (n = 57)	1.38 ± 0.37	1.46 ± 0.72	1.27 ± 0.44
Gleason score			
2–6 (n = 46)	1.32 ± 0.46	1.35 ± 0.27	1.23 ± 0.28
7–10 (n = 35)	1.37 ± 0.50	1.45 ± 0.68	1.34 ± 0.53

The values are expressed as means ± SD.
d, day; m, month.

TABLE IV. Pre- and Postoperative Characteristics of Transformed α2-M Plasma Levels (μg/ml) in 81 Patients Undergoing Radical Prostatectomy

	Preoperative (−2d)	3 months postoperative (+3m)	6–12 months postoperative (+12m)
Prostatectomy patients (n = 81)	2.80 ± 1.40	3.51 ± 2.50	3.60 ± 0.48
T1-2N0M0 (n = 38)	2.62 ± 1.19	3.96 ± 3.18	2.88 ± 1.65
T3-4N0M0 (n = 39)	2.84 ± 1.49	3.08 ± 1.56	4.33 ± 6.70
TxN1M0, TxN0M1, TxN1M1 (n = 4)	3.44 ± 2.02	2.79 ± 1.36	2.96 ± 1.88
Androgen deprivation			
Yes (n = 57)	2.87 ± 1.33	3.87 ± 2.81	3.47 ± 3.62
No (n = 24)	2.52 ± 1.49	2.56 ± 0.89	3.84 ± 6.97
Gleason Score			
2–6 (n = 46)	2.75 ± 1.31	3.64 ± 2.47	3.88 ± 5.84
7–10 (n = 35)	2.78 ± 1.49	3.26 ± 2.50	3.19 ± 3.02

The values are expressed as means ± SD.
d, day; m, month.

DISCUSSION

Elevated TGF-β1 level seems to be associated with a variety of progressive and clinically relevant diseases. TGF-β1 inhibits the growth of most non-malignant cells, particularly of epithelial origin, and induces cell death in normal prostate cells. The induction of apoptosis in the prostate after androgen-ablation therapy is thought to be mediated by TFG-β1. Unfortunately, however, many malignant cells are resistant to TGF-β1-mediated growth inhibition. This is probably due to a downregulation or mutation of TGF-β1 receptors, which may partially explain the progression of the disease despite of high TGF-β1 plasma levels in cancer patients [2]. On the other hand, TGF-β1 might promote PCa growth and metastasis via its stimulating effect on angiogenesis and via immunosuppression [6,7].

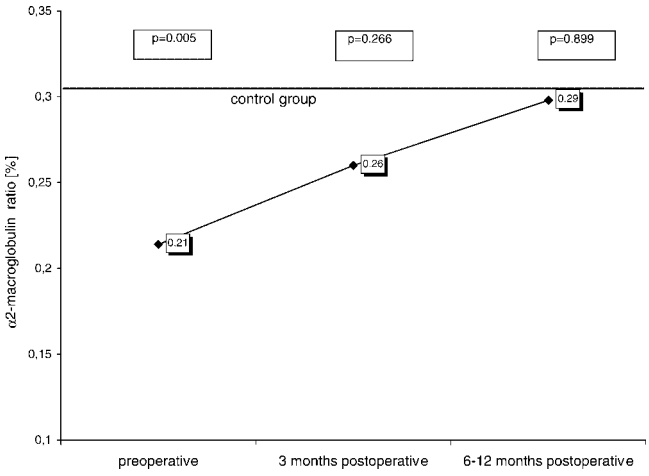

Fig. 3. Pre- and postoperative characteristics of the α2-macroglobulin ratio in 81 PCa patients compared to healthy individuals. The ratio of α2-M was determined as the fraction of transformed α2-M (mg/ml) compared to total α2-M concentration (mg/ml) given as a percentage (ordinate). The ratio (mean ± SD) was measured in the plasma obtained preoperative (−2d) (0.21 ± 0.10) and postoperative at 3 months (+3m) (0.26 ± 0.19) and 6 – 12 months (+12m) (0.29 ± 0.53) after prostatectomy, respectively. The α2-M ratio of the control group was 0.31 ± 0.26 (solid horizontal line). Control group values versus preoperative value (−2d) ($P = 0.005$).

In the present study, we observed statistically significant differences in TGF-β1 levels between patients with PCa and healthy men without cancer; furthermore, we found elevated levels of TGF-β1 in four patients with local and remote metastases. This is in accordance with the observation of Shariat et al. [8] who found that TGF-β1 is markedly increased in men with PCa metastatic to bone and lymph nodes. Thus, even in the absence of pathological and clinical evidence of metastases, the preoperative TGF-β1 level might be a predictor of progression [9].

Androgen ablation has been reported to induce an enhanced production of TGF-β1 in human PCa [2]. We found decreased TGF-β1 levels in the plasma of androgen-deprived patients; this difference between treated and non-treated groups disappeared approximately 12 months after tumor dissection. It remains to be proven whether this may be an early indication of an androgen-independent stage. Noteworthy, testosterone was found to induce an overexpression of TGF-β1 in many other cells, including mammary epithelial cells [10] and human adrenocortical cells [11]. Apparently, the elevated TGF-β1 levels in non-deprived patients may originate from responsive prostate cells, as androgens were shown to cause a dose-dependent increase in active TGF-β1 released from PCa cells [12] and from other organs or tissues distant to the prostate. Our observation of sustained high TGF-β1 levels after prostatectomy argue in favour of the suggestion that elevated TGF-β1 levels are generated by host factors

such as distant organ responses to invasion rather than as a result of tumor-induced metabolic changes in the prostate.

For the first time, we could demonstrate an association between TGF-β1 levels and BMI in PCa patients. A similar correlation has been recently shown for hypertensive patients and indicates that adipose tissue may be an important determinant of TGF-β1 levels [13]. To verify these preliminary results, more comprehensive investigations within a larger cohort should be conducted; although, epidemiological studies have shown that a Western diet is associated with an increased incidence of PCa [14] and fat restriction has been shown to inhibit the growth of transplanted PCa cells in rodents [15]. As fat-related hormones, such as leptin, exert different effects on various human tissues and cells, it can be anticipated that these hormones may be also involved in BMI-related TGF-β1 expression.

The activity and bioavailability of TGF-β1 is regulated by TGF-β1 binding plasma proteins. Among them, α2-M binds this growth factor with high affinity [16]. Neutralization of TGF-β1 by transformed α2-M was found to abrogate the immunosuppressive activity of human breast cancer cells [17]. Because α2-M targets a variety of cytokines and growth factors, it is assumed to play an important role in their homeostasis. Recently, we demonstrated that the plasma concentration of α2-M dramatically decreases with increasing age, which would have a strong impact on the α2-M-mediated clearance mechanism in humans [18].

Here, we observed significantly decreased α2-M levels in PCa patients compared to age-matched controls. This is in accordance with Kanoh et al. [19] who reported a loss of α2-M in PCa patients with bone metastases.

Since the levels of both TGF-β1 and α2-M appear to be inverse but reliable predictors of PCa, we tested the relationship between the two proteins in all cohorts and at different times of analysis. However, we failed to find any significant association, indicating that these two proteins are independently regulated.

Assuming that altered levels of TGF-β1 and α2-M are involved in the pathogenesis of PCa, therapeutic strategies should aim at decreasing the TGF-β1 levels and increasing the α2-M levels in patients. As far as PSA is concerned, this protein is known to stimulate IGF-I-mediated growth of prostate cells by degrading the IGF-binding protein, IGFBP-3 [20], the reduction of PSA is suggested to stimulate immune responses [21], and enzymatically active PSA was found to have growth factor activity to human osteosarcoma cells [22]. Based on these in vitro results, it seems reasonable to assume that binding and rapid elimination of PSA might possibly improve the prognosis of PCa. As α2-M is the main inhibitor and clearance factor of PSA, a reduced concentrations of α2-M might thus be relevant to disease development. On the other hand, PSA was found to exert anti-angiogenic effects by stimulating angiostatin production [23]. These seemingly opposing results show that more substantial studies are required to expose the different facets of the PSA action in vivo.

It was recently shown that the application of proteinases triggers the clearance of TGF-β1 in blood and tissue [24,25]. As demonstrated in Figure 3, the fraction of proteinase-complexed α2-M is decreased in patients with PCa but is almost completely restored within 12 months postoperation. This could mean that in PCa there is a lack of proteolytic activity in plasma, possibly impairing the α2-M-mediated clearance of TGF-β1, and of other growth factors as well. The dramatic loss of α2-M in elderly people, which is even more pronounced in PCa patients, may further impair the clearance function of the inhibitor [18].

Altogether, it seems valuable to prove the administration of proteolytic enzymes in prostate patients to stimulate clearance of TGF-β1 and probably of other pathogenic factors such as IGF-I and leptin, which also bind to α2-M and are considered to be risk factors for PCa [26,27].

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