Chapter 4

Thrombin-activatable Fibrinolysis Inhibitor levels in prostate cancer patients

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Summary

Prostate cancer has historically been associated with hemostatic abnormalities. High plasma levels of markers of fibrinolysis indicate that the plasminogen activation system is activated in patients with prostate cancer. Thrombin-activatable fibrinolysis inhibitor (TAFI) is a B-type carboxypeptidase present in blood that can inhibit plasminogen activation. We hypothesized that TAFI may regulate activation of the fibrinolytic system in prostate cancer patients. This study was undertaken to investigate whether TAFI antigen and activity levels are influenced in patients with prostate cancer. We found that circulating plasma antigen and activity levels of TAFI are normal in prostate cancer patients and independent of the presence of metastases. Furthermore, TAFI levels were not associated with progression of the disease or with the presence of thrombosis. Our results suggest that TAFI does not play a major role during prostate cancer.
Introduction

It is well recognized that cancer patients have a high incidence of hemostatic complications such as disseminated intravascular coagulation, venous thromboembolism and hemorrhage. It has been reported that advanced prostate cancer is responsible for up to 25% of incidences of disseminated intravascular coagulation, presenting as a bleeding tendency with or without venous thrombosis. There is also substantial evidence that the hemostatic system is involved in cancer progression and the formation of metastases. For example, proteins involved in maintaining hemostasis also regulate angiogenesis to support tumor growth. Clinical studies investigating the role of the hemostatic system in prostate cancer have indicated the presence of abnormal levels of proteins of the coagulation and fibrinolytic systems such as antithrombin III, prothrombin fragment 1 and 2, thrombin-antithrombin III (TAT) complexes, plasmin-α2-antiplasmin (PAP) complexes, plasminogen activator inhibitor (PAI-1) and fibrin degradation products. These abnormalities often reflect tumor malignancy and correlate with disease prognosis. The prevalence of these circulating markers is rather low in patients with primary cancer but high in patients with advanced prostate cancer. Adamson and colleagues have shown that the levels of fibrin degradation products correlate with bone scan positivity in prostate cancer patients. Thus, activation of the fibrinolytic system is seen in patients with prostate cancer.

Proteolytic enzymes involved in fibrinolysis are the serine proteases of the plasminogen activation system, tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA) and plasmin. Both tPA and uPA generate plasmin by proteolytical cleavage of its zymogen plasminogen. Plasmin cleaves its substrates behind a lysine or arginine, which then become carboxy-terminal. Such carboxy-terminal basic amino acid residues in fibrin provide high affinity binding sites for plasminogen and facilitate efficient plasminogen activation by tPA and uPA.

Thrombin-activatable fibrinolysis inhibitor (TAFI), also known as carboxypeptidase U, R or plasma procarboxypeptidase B (EC 3.4.17.20) links the coagulation and fibrinolytic systems and regulates fibrinolysis in vitro and in vivo. When converted into its active form by thrombin, plasmin or trypsin, TAFI is a carboxypeptidase B-type enzyme that specifically cleaves carboxy-terminal lysine or arginine residues from proteins. TAFI-mediated removal of these carboxy-terminal amino acids from fibrin attenuates efficient plasminogen activation into plasmin. Thrombin-mediated activation of TAFI is stimulated 1250-fold by the endothelial cell receptor thrombomodulin. Circulating TAFI antigen levels broadly vary between different healthy individuals and correlate with TAFI activity and clot lysis time. Recently, several studies have been reported that indicate an association between elevated TAFI levels and thrombosis. Reduced TAFI activity, but not TAFI antigen, in patients with acute promyelocytic leukemia was possibly due to proteolytic inactivation by excess fibrinolytic activity.

In the past, ε-aminocaproic acid (εACA), a lysine analogue that inhibits plasminogen activation, has been successfully used for the treatment of hemostatic complications in
prostate cancer patients. This indicates that the plasminogen activation system and particularly carboxy-terminal lysines play an important role in these complications. We hypothesize that TAFI may influence cancer growth and/or hemostatic complications in patients with prostate cancer. Here we measured the plasma TAFI antigen and TAFI activity levels in patients with primary and metastasized prostate cancer.

Materials and methods

Patients and controls
We collected samples from 119 patients with prostate cancer. For controls, plasma samples were collected from 22 males with no evidence of cancer. Blood was collected by venepuncture in 3.2% (w/v) sodium citrate (1:10) (Venoject, Terumo Europe N.V. Leuven, Belgium). The samples were centrifuged for 15 minutes (2000g) and the supernatant was stored at −80°C until use. In all samples from cancer patients routine prostate specific antigen (PSA) was determined.

Measurement of TAFI antigen and activity
TAFI antigen was determined with an enzyme-linked immunosorbent assay (ELISA) as has been described. TAFI activity in plasma was measured as follows. Plasma was diluted 5 times in 50 mM Hepes, pH 7.4. Activation of TAFI was performed at room temperature by the addition of 5 µl of plasma to 50 µl activation buffer containing 50 mM Hepes, pH 7.4, 40 nM rabbit lung thrombomodulin (American Diagnostica, Greenwich, CT), 10 nM thrombin (Enzyme Research Laboratories, South Bend, IN), 20 mM CaCl₂ and 8 mM hippuryl-Arg (Bachem, Bubendorf, Switzerland), a substrate for activated TAFI. After 30 minutes, the reaction was stopped by 50 µl 1M HCl. After the addition of internal standard (20 µl of 22.5 µM O-methylhippuric acid) cleaved substrate was extracted with 300 µl ethylacetate, air-dried, dissolved in 150 µl 50 mM K₃PO₄, 20% acetonitril, pH 3.5 and analyzed using high performance liquid chromatography (HPLC; XTerra MS C18 3.5 µm, 4.6 x 100 mm column, Waters Chromatography BV, Ettenleur, The Netherlands). To distinguish between constitutive active carboxypeptidase (carboxypeptidase N) and active TAFI in plasma, carboxypeptidase activity sensitive to a potato carboxypeptidase inhibitor (CPI) was considered to be TAFI activity.
Results and discussion

Patients
Hundred and nineteen prostate cancer patients and twenty-two age-matched controls without evidence of cancer were investigated. Hundred and four of the patients had metastases to distant organs and fifteen patients had cancer limited to the prostate. The mean age in the group of non-metastatic prostate cancer patients was 64 yr (range, 44 - 74 yr) and in the group of patients with metastases 67 yr, (range, 42 - 87 yr). The control population included patients with a mean age of 60 yr (range, 40 - 88 yr). The median serum PSA levels in the patients with prostate cancer were 154 ng/ml (range, 0 – 9513 ng/ml) in the group with metastases and 0.8 ng/ml (range, 0 – 30 ng/ml) in the non-metastatic patient group.

TAFI levels
TAFI antigen and activity levels in the control group were normalized and considered 100%. When expressed as a percentage of that in controls (100 ± 3%, n=22), the mean TAFI antigen level in primary prostate cancer patients was 101 ± 3% (mean ± SEM, n=15) and 101 ± 1% (n=104) in metastasized prostate cancer patients (Figure 1a). Compared to controls (100 ± 3%, n=22), TAFI activity in plasma of primary prostate cancer patients was 99 ± 3% (mean ± SEM, n=14) and 102 ± 2% (n=104) in patients with metastasized prostate cancer (Figure 1b). These results indicate that TAFI levels did not change in prostate cancer patients. We hypothesized that TAFI may only be significantly affected in prostate cancer patients with high circulating levels of an indicator of prostate cancer activity, prostate specific antigen (PSA). However, TAFI antigen and activity did not correlate with PSA in patients with local tumor burden or metastases (not shown). In control subjects, TAFI antigen and activity will be highly correlated with each other. In other words: TAFI activity is fully determined by the amount of TAFI antigen. We investigated whether prostate cancer influences the relation between TAFI antigen and TAFI activity. We found that, although TAFI activity correlated with TAFI antigen in all groups, this correlation was strongly reduced in patients with metastases (Pearson correlation in control: 0.6; primary prostate cancer: 0.6; metastatic prostate cancer: 0.4) (Figure 2a-c). This observation could not be explained by disease progression (PSA levels). TAFI antigen or activity levels were not associated with the incidence of thrombosis.
TAFI antigen and activity levels were measured in plasma of prostate cancer patients and controls. TAFI antigen and activity levels are expressed as percentage of control. TAFI antigen (A) and activity (B) levels are not different between patients with primary prostate cancer, metastasized prostate cancer and controls.

Plasma levels of TAFI antigen and TAFI activity are associated. (A-C) TAFI activity correlated with TAFI activity in all groups. However, when compared to controls and patients with primary prostate cancer, antigen/activity correlation was decreased in patients with metastases (control: 0.6; primary prostate cancer: 0.6; metastatic prostate cancer: 0.4).
Discussion

Abnormalities of the coagulation and fibrinolytic systems are frequently found in cancer patients. Here we show that circulating plasma levels and activity of a regulator of fibrinolysis, TAFI, are normal in prostate cancer patients with or without evidence of metastases.

Our findings are somewhat unexpected. Based on the fact that the coagulation (thrombin) and/or fibrinolytic (plasmin) system are induced in prostate cancer patients, we expected that TAFI would be activated and consumed. Alternatively, TAFI levels could be negatively affected by inactivation of the enzyme through cleavage by increased plasmin present in prostate cancer patients. However, we did not observe abnormal TAFI antigen or activity levels in these patients. In the past, only one other similar study has been reported in patients with cancer. Although patients with acute promyelocytic leukemia (APL) had normal TAFI antigen levels, Meijers et al. 32 have shown that a large amount of TAFI circulated in a form that could not be activated. The authors suggest that hyperfibrinolysis is responsible for the decreased activity of TAFI in these patients. When compared to prostate cancer, APL is associated with an even higher activation state of the fibrinolytic system. Apparently, the relatively low amount of activated plasminogen in prostate cancer patients, compared to patients with APL, does not affect TAFI.

Taken together, the growth and metastasis of prostate cancer and the hemostatic complications that are often observed in prostate cancer patients are not associated with changes in TAFI. Moreover, prostate cancer does not seem to affect the activation of circulating TAFI.

Acknowledgements

The authors thank Dr. H.J. Bloemendal, Dr. P.O. Witteveen, Dr. A. de Graeff, Dr. S. Radema, Dr. P. Quarles van Ufford and Dr. G. Groenewegen for providing blood samples and the nurses of the daycare of medical oncology (UMCU) for blood sampling. This study was supported in part by grant (UU 1999-2114) of the Dutch Cancer Society (EEV/MFBGG) and the Fischer Stichting (AR).

References


