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Cellular fibronectin in patients with transitional cell carcinoma of the bladder

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Abstract Various tumor markers for transitional cell carcinoma (TCC) of the bladder have been described, but none of them are used in clinical routine. Fibronectin, a glycoprotein, seems to play a very important role in both the progression and invasion of cancer. The aim of this study was to evaluate cellular fibronectin (cFN) in the urine and blood of patients with TCC of the bladder and to determine its possible role as a tumor marker and prognostic factor. Morning urine samples and blood were collected from 20 patients (8 women, 12 men, mean age 69.9 years) before they underwent transurethral resection of bladder tumors (TURB). Twenty patients (10 women, 10 men, mean age 63.4 years) with nonmalignant urological disorders were recruited as the control group. Determination of cFN in plasma and urine was performed by using a newly developed time-resolved fluorescence immunoassay (TRFIA). Patients with nonmalignant diseases had mean cFN plasma levels of 404 ng/ml (range 181–746 ng/ml). Patients with TCC of the bladder showed significantly higher cFN plasma levels of 686 ng/ml (range 274–1999 ng/ml, $p < 0.05$). Subdivided according to the TNM system, muscle-invasive bladder tumors ($n = 5$) demonstrated higher cFN plasma levels (mean 944 ng/ml) than superficial bladder tumors ($n = 15$, mean 463 ng/ml). There were no differences of plasma cFN concentrations concerning tumor grade and also no differences in urine levels between the different groups. We found a significant difference ($p < 0.04$) of cFN plasma levels between patients with TCC of the bladder and the control group. The difference in cFN plasma levels between pTa/pT1 and ≥pT2 tumors indicates a clinically useful potential of this tumor marker for preoperative staging and postoperative follow-up. Our data underline the important but still unclear role of cFN as a tumor marker in TCC, and this will be the focus of future studies.

Keywords Fibronectin · Bladder cancer · Biological marker · Prognostic factor

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Introduction

Transitional cell carcinoma (TCC) is the second most common urological tumor with an incidence of 40 newly diagnosed cases per 100,000 inhabitants per year in Germany [8]. Initially, the majority of TCC present at a superficial stage, but after primary treatment, TCC will recur in 70% of the cases within 2 years [7]. Additionally, 10%–20% will progress or metastasize during the future clinical course [6]. For effective treatment, early detection is mandatory, and various markers have been described to facilitate screening in high-risk groups [1, 15, 20, 22]. However, due to their low sensitivity and specificity, none of these markers has come into clinical use. Currently, only urinary cytology has been proven to detect high-grade bladder cancer with a high sensitivity and specificity. However, no reliable and reproducible markers are available which predict the presence of muscle invasion and/or metastases. Therefore, it appears to be of significant clinical interest to identify reliable serological and urinary markers for the early detection of primary TCC, for adequate monitoring following primary therapy, and for use as a prognostic factor.

Fibronectin, a glycoprotein of 440 kDa, plays an important role in the inhibition of cellular attachment and promotion of migration, thereby facilitating invasion of malignant tumors mediated by specific receptors and growth factors [9, 13]. Additionally, fibronectin is

involved in each phase of wound healing. Fibronectin is found on the surface of fibroblasts and promotes wound healing by interlacing with fibrin and thus fixates fibroblasts via activation by factor XIIIa. Another mechanism is its ability to interact with collagen fibers and anchor fibroblasts [3]. The main percentage of total fibronectin in blood is synthesized by hepatocytes, whereas cellular fibronectin (cFN), a distinct form derived from other cells, is found in much smaller amounts. cFN is distinguished from the hepatocyte-derived form by having a specific extra domain sequence (EDA), which originates by differential splicing of the FN precursor mRNA. An insoluble matrix form of fibronectin has been demonstrated along the urothelial tissue [3]. A recent study demonstrated that fibronectin tissue levels were significantly elevated in TCC of the human bladder [10]. Initial results demonstrated higher urinary levels of oncofetal fibronectin in patients with TCC [21]. Another study used bladder tumor fibronectin (BTF) urinary levels for follow-up. They concluded that in patients with no evidence of BTF after primary therapy, the number of cystoscopies could be reduced during follow-up [17]. Intravesical bacillus Calmette-Guérin (BCG) effects seem to be influenced by fibronectin. It was shown that intravesical adhesion of the bacteria is inhibited by pretreatment with fibronectin, but its role as a marker for selecting patients for BCG therapy is still unclear [4, 13, 16, 18]. Malmström and co-workers measured urinary fibronectin levels in patients suffering from TCC. They concluded that low urinary fibronectin levels predict a better response to BCG therapy [14]. So the determination of fibronectin in patients with TCC may prove to be of clinical value. For measuring cFN levels in urine and plasma, we have used a highly sensitive, time-resolved fluorescence immunoassay (TRFIA), using a monoclonal antibody which is specific for cFN [11, 12]. The aim of the present study was to evaluate the clinical value of cFN in urine and plasma as a tumor marker in the diagnosis of TCC and to determine a possible relationship between the cFN levels and the histopathological stage.

Patients and methods

To evaluate the value of cFN in the diagnosis of TCC, morning urine samples and blood were collected from 20 patients (8 women, 12 men, mean age 69.9 years) before they underwent transurethral resection of TCC. Physical examination, chest X-ray, intravenous urography, and abdominal sonography were used for the clinical staging. Twenty patients (10 women, 10 men, mean age 63.4 years) with different nonmalignant urological disorders (i.e., hydrocele testis, ureteropelvic junction obstruction, stone disease, benign prostatic hyperplasia, urinary incontinence) were recruited as the control group. Patients with other malignancies in their medical history were excluded.

Blood samples were collected in ethylene diamine tetra-acetic acid (EDTA)-containing tubes and processed for plasma within 3 h. After centrifugation (2000 rpm, 10 min) and removal of supernatant plasma, the samples were stored at –70°C. Additional morning urine samples were collected and stored at –70°C until measurement (maximum of 3 months).

cFN was determined by using a highly sensitive TRFIA specific for the cellular form of fibronectin. This sandwich-type assay is based on a cFN-specific antibody (mouse, monoclonal) immobilized on standard enzyme-linked immunosorbent assay (ELISA) microwells. After application of standards and samples, a polyclonal rabbit FN-specific antiserum was used as the secondary antibody. For the final detection step, a modified biotin-streptavidin method with measurement of an Eu^{3+} chelator was used [11, 12]. The pathohistologic diagnosis of the main tumor specimen was done by the Department of Pathology at the Philipps University Marburg Medical School according to the UICC classification of 1997 [19]. Measurement of the plasma samples was always performed by the same person. Statistical analysis was performed using the nonparametric Mann-Whitney U-test to compare the results of TCC patients with controls. The Kruskal-Wallis ANOVA test was used to analyze the differences between the different groups and subgroups (SPSS Software). Values of $p < 0.05$ were accepted as statistically significant.

Results

The intra-assay variance was evaluated by 20-fold determination of one sample. The coefficient of variance was 4.7%. The interassay variance was determined on five different days. The coefficient of variance was 7.2%. Patients with TCC had mean cFN plasma concentrations of 686 ng/ml (range 274–1999 ng/ml). The control group demonstrated mean cFN concentrations of 404 ng/ml (range 181–746 ng/ml). The difference (Mann-Whitney U-test) between the two groups was statistically significant with $p < 0.04$ (Fig. 1).

Subdividing patients with TCC according to the UICC TNM system of 1997 [19], muscle-invasive bladder tumors ($n = 5$) demonstrated higher cFN plasma levels (mean 944 ng/ml, SD ± 664 ng/ml) vs superficial bladder tumors ($n = 15$, mean 463 ng/ml, SD ± 274 ng/ml),

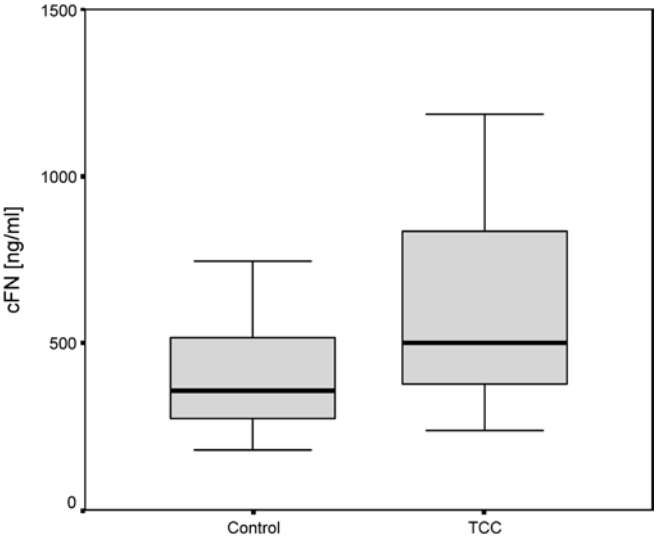

Fig. 1 Concentrations of cellular fibronectin (cFN) plasma levels in patients with transitional cell carcinoma (TCC) of the bladder ($n = 20$) and a control group with benign urological diseases ($n = 20$). A significant difference between this two groups was detectable ($p < 0.04$, Mann-Whitney U-test)

but the difference was not significant ($p = 0.058$, Mann-Whitney U-test; Fig. 2). Comparing the control group, patients with superficial bladder tumors, and those with muscle-invasive bladder tumors, no significant differences between these three groups could be detected ($p > 0.05$, Kruskal-Wallis ANOVA test; Table 1). There were no differences of plasma cFN concentrations with regard to tumor grade. The concentrations of cFN in morning urinary samples were not significantly different between groups.

Discussion

The aim of the study was to evaluate for the first time the clinical utility of cFN in urine and plasma of patients suffering from TCC of the bladder using a recently described assay [11, 12]. Fibronectin, a multifunctional glycoprotein, facilitates the invasion of malignant tumors by interacting with receptors and growth factors, which leads to the inhibition of cellular attachment and the promotion of cellular migration. Additionally, fibronectin supports wound healing by interacting with fibrin and collagen, and an insoluble matrix form of fibronectin was found along the urothelian tissue [3, 9, 23]. Recent studies on the expression of fibronectin in bladder cancer tissue revealed diverse results. An explanation for this may be the application of different techniques, immunohistochemical staining versus enzyme immunoassay [5, 10]. In contrast to other investigations, we found no elevated cFN levels in the morning urine samples of patients with TCC [4, 10, 17, 21]. Laufer et al. detected increased urinary fibronectin levels after transurethral resection of noninvasive bladder tumors [13]. Before transurethral resection, no elevation of urinary fibronectin was noticed, and in most patients, levels normalised within 2 weeks after the operation so that a possible role of fibronectin in the failure of BCG therapy was concluded. The postinvasive alteration may be caused by the activation of fibronectin in urothelian wound healing, and the amount of measured fibronectin may depend on the resected tumor size and urothelian defect. The definitive role of fibronectin in selecting patients for BCG therapy in case of superficial TCC is still unclear in the face of contradictory study findings [4, 5, 14]. However, in our opinion, the contrary findings of urine fibronectin levels can be caused by the different assay methodologies used and their problems of comparability. On the one hand, different types of fibronectin (cFN versus total fibronectin) were measured with the different analytical methods. On the other hand, we cannot exclude the presence and determination of abnormal fibronectin forms in urine, as has been demonstrated in other cell systems [2]. Perhaps these abnormal forms may be responsible for the higher urinary levels. To our knowledge, this is the first study involving the determination of cFN in plasma from patients suffering from TCC of the bladder. Statistical analysis with the Mann-Whitney U-test showed significantly higher plasma levels of cFN in TCC in comparison with the control group ($p < 0.04$). This result can be regarded as support for a potentially valuable role of cFN as a tumor marker for TCC. In cooperation with other diagnostic tools like cystoscopy and urinary cytology, the determination of cFN plasma levels perhaps offers an opportunity to differentiate unclear bladder findings before therapy. In addition, we found lower plasma levels of cFN in patients with superficial (pTa/pT1) than in those with muscle-invasive (≥pT2) TCC of the bladder. Due to the small patient numbers, these results were not statistically significant. However, our data indicate a potential relationship between cFN plasma levels and tumor progression.

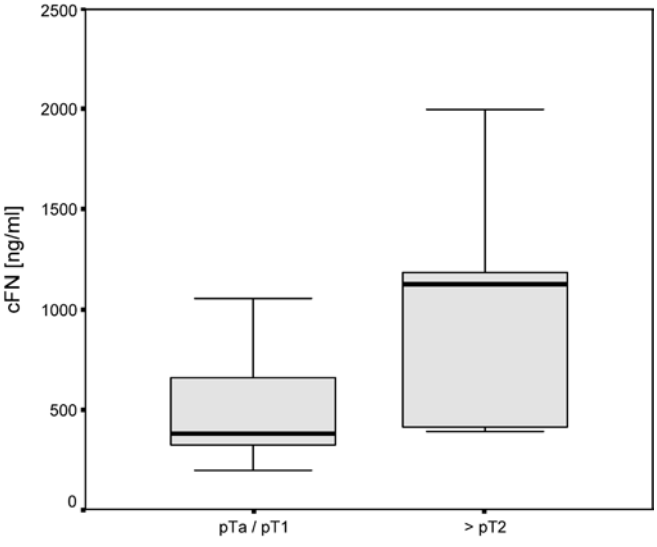

Fig. 2 Concentrations of cFN plasma levels in patients with superficial ($n = 15$) and muscle-invasive TCC of the bladder ($n = 5$). A significant difference between these two groups was not detectable ($p = 0.058$, Mann-Whitney U-test)

Table 1 cFN plasma concentrations of the control group and patients with pTa/pT1 and ≥pT2 bladder tumors. A significant difference between the three groups was not detectable ($p > 0.05$, nonparametric Kruskal-Wallis ANOVA test)

	Control ($n = 20$)	pTa/pT1 ($n = 15$)	≥pT2 ($n = 5$)
cFN (ng/ml)	404	463	944
±SD	161	274	664

In summary, we found significantly elevated plasma cFN in patients suffering from TCC of the bladder compared with normal individuals. In a small group of patients with muscle-invasive TCC, elevated but not significantly higher levels were found in comparison with superficial TCC. Our data underline the important but still unclear role of fibronectin as a tumor marker in TCC. Further studies with a larger number of patients have to be performed to work out the definitive role of cFN as a tumor marker predicting muscle-invasive

disease or metastatic progression in TCC of the human bladder.

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