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Impact of serum basic fibroblast growth factor on prognosis in human renal cell carcinoma

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Abstract

Renal cell carcinoma is often characterised by extensive vascularity and angiogenic factors may be of importance for disease progression. Using a sandwich enzyme immunoassay, basic fibroblast growth factor (bFGF) was analysed in the sera from 206 patients with renal cell carcinoma before the initiation of therapy. The median bFGF level was 3.0 pg/ml (range <1.0–70.9 pg/ml). The serum levels were significantly correlated to tumour stage and nuclear grade. Patients with tumour thrombus to the renal or the inferior caval vein had significantly higher serum bFGF levels compared with those with non-invading tumours ($P=0.007$). Patients with serum bFGF levels above 3.0 pg/ml had a worse prognosis, compared with those with lower levels ($P=0.001$). Furthermore, patients with tumours with vein invasion had a worse prognosis compared with those without invasion. After multivariate analysis, only tumour stage and grade remained as independent prognostic factors.

Keywords: Renal cell carcinoma; Basic fibroblast growth factor; Angiogenic factor; Stage; Nuclear grade; Stability; Prognosis

1. Introduction

Basic fibroblast growth factor (bFGF) is an 18 kD protein produced by several different types of cells and has a strong affinity for heparin. It is mitogenic for vascular endothelial cells [1,2] and enhanced expression of bFGF has been observed in wound healing, in patients with ischaemia [3], as well as in a variety of malignant tumours [4]. Furthermore, bFGF may also be involved in the resistance of tumour cells to chemotherapy [5].

Renal cell carcinoma is often characterised by extensive vascularity, a point that is crucial for the metastatic procedure [6]. Conflicting results concerning the impact of microvasculature on prognosis in renal cell carcinoma have been presented [7,8]. bFGF was first demonstrated in renal cell carcinoma, by Mydlo and associates [9] using western blot analysis, and later by Eguchi and associates [10] and Nanus and associates [11]. Only approximately 15% of the tumours showed positive immunohistochemistry staining for bFGF [11], but these tumours were associated with a more dismal prognosis. bFGF has also been demonstrated in cell lines and in transplanted renal cell carcinomas [12,13], as well as in the urine from mice with transplanted tumours [14] and from patients with urological tumours [4,15].

There are several reports on serum bFGF from a relatively limited number of patients with renal cell carcinoma [16–23]. Some of these reports show increased levels in patients with renal cell carcinoma compared with healthy controls [18,20]. Furthermore, patients with metastases had higher levels compared with patients with non-metastatic disease [20]. The serum level of bFGF, however, seemed not to be an independent prognostic factor. The purpose of this study was to evaluate serum bFGF in relation to tumour stage, vascular invasion and prognosis in an extended group of patients with renal cell carcinoma.

2. Patients and methods

Serum samples from 206 patients with renal cell carcinoma was collected prior to therapy, and stored at

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−80 °C for later analysis. There were 122 (59%) male and 84 (41%) female patients, with a median age of 66 years (range 28–85 years). Serum sampling was performed, after patients' informed consent, from consecutive patients from 1982 through to 1997 at the Department of Urology, University Hospital in Umeå, Sweden. Sera from 10 patients with benign renal cysts were used as controls. bFGF was assayed in duplicate in 86% of the samples (single analysis was performed in 28 patients due to shortage of material) with a sandwich enzyme immunoassay method (Quantikine, DFB00, R&D Systems, Minneapolis, MN, USA), with a detection limit of 1.0 pg/ml.

Following clinical investigation, including computerised tomography the tumour stage was assessed according to TNM [24]. 195 (95%) patients were operated upon with radical, and 3 (1%) with partial nephrectomy, 8 (4%) patients had palliative treatment with medroxyprogesterone acetate, interferon or arterial occlusion. Maximal tumour diameter was based on direct measurement of the surgical specimen or from the computerised tomographies. Nuclear grading was performed according to Skinner and associates [25]. Tumour invasion to renal or the inferior caval vein was assessed by macro- and microscopic analysis and by radiography. No patients with severe cardiac disease or peripheral ischaemic disease were included in the study.

The patients were followed according to clinical routine. Relapse of disease was registered. In case of death, the cause was based on medical records and death certificates. Cause-specific survival was calculated from the time of admission, according to the method of Kaplan and Meier, and evaluated using the log rank test. For statistical analysis, the Mann–Whitney, Kruskal–Wallis and Fisher's exact tests were used.

3. Results

bFGF was detected in 148 of the 206 (72%) sera from patients with renal cell carcinoma. The frequency of detectable levels was higher in samples stored 1–8 years, compared with those stored 9–16 years, 85 versus 61% ($P=0.004$; Fisher's Exact test), indicating a certain amount of inactivation of bFGF during storage. The median level was 3.0 pg/ml (range <1.0–70.9 pg/ml) significantly different from that of the 10 patients with benign renal cysts, <1.0 pg/ml (range <1.0–4.4 pg/ml) ($P=0.03$). No significant difference in the bFGF level was found related to gender or age. The relationships of serum bFGF to tumour stage and nuclear grade are presented in Fig. 1 and Table 1. For both, a positive correlation was found ($P<0.001$). When serum bFGF was related to tumour diameter in stage I–II, using linear correlation, no significant relationship was observed.

Tumour thrombus in the renal, and the inferior caval vein was found in 49 and 26 patients, respectively. As shown in Table 2, serum bFGF levels were significantly higher in patients with tumour thrombus, compared with those without vein invasion ($P=0.007$).

68 patients had distant metastases, stage IV disease, at the time of diagnosis. A majority, 38, of them had metastases to multiple organs, but 18 patients had lung metastases only. When serum bFGF was assessed in relation to the organ of metastases, we were unable to find any significant differences in bFGF levels, due to site of the metastases. Nor did we find any differences in the serum bFGF levels in stage I–II patients who later developed metastases, due to localisation of the secondary tumours.

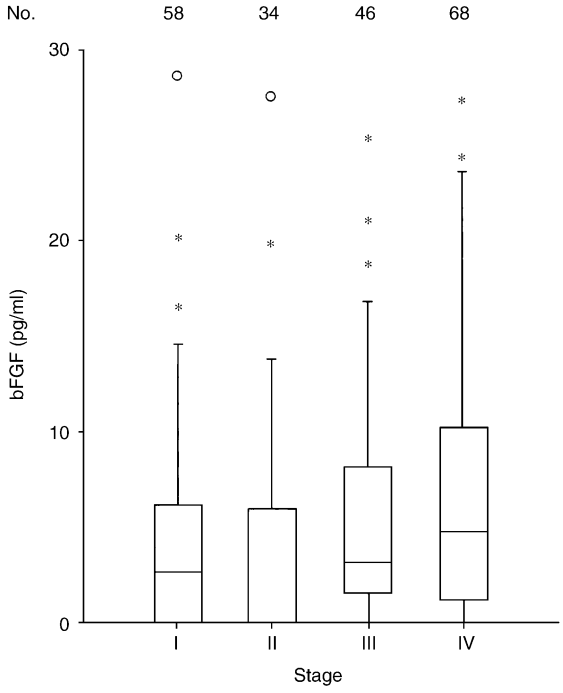

Fig. 1. Box-and-whisker plot of serum basic fibroblast growth factor (bFGF) in relation to tumour stage in renal cell carcinoma. The boxes represent the 25th to the 75th percentile. * and ° represent values more than 1.5 and 3 boxlengths from the 75th percentile, respectively.

Table 1
Serum basic fibroblast growth factor (bFGF) in relation to nuclear grade in patients with renal cell carcinoma

Grade	No.	Not detectable (<1.0 pg/ml)	bFGF (pg/ml)	No. ⩾5 pg/ml	P value[a]
		No. (%)	Median (range)		
1–2	47	18 (38)	1.4 (<1–38)	14	
3	102	28 (27)	3.2 (<1–34.1)	39	<0.001
4	55	12 (22)	4.8 (<1–70.9)	27	

[a] Kruskal–Wallis test.

The median follow-up time for patients that are alive was 112 months (range 32–210 months). During the follow-up, 109 patients died of renal cell carcinoma, and 36 of intercurrent diseases. When cause-specific survival was analysed, patients with serum bFGF levels >3.0 pg/ml had a significantly worse prognosis compared with those with lower levels ($P=0.001$), as shown in Fig. 2.

Evaluation of clinical parameters in relation to prognosis is shown in Table 3. Tumour stage, grade, vein invasion and serum bFGF were related to prognosis. However, in the multivariate analysis, shown in Table 4, only tumour stage and grade were independent prognostic factors, while tumour vein invasion and serum bFGF level added no information concerning prognosis.

4. Discussion

bFGF is produced by several different types of cells, and hypoxia is probably the strongest inducer [1]. Support for this hypothesis comes from results on cells cultured under hypoxic conditions [26], as well as the increased expression of bFGF observed in patients with ischaemic diseases [3,27]. Increased expression of bFGF in cancer diseases might reflect hypoxia in the tumour tissue. Necrosis being the final result of hypoxia might also lead to an increased release as a result of tumour-tissue degradation.

The stability of bFGF has been evaluated before [28,29], and during storage at −80 °C a decline in the potency of bFGF was found. This observation is supported by our results, that show a higher frequency of non-measurable levels of bFGF in samples stored for more than 9 years, compared with samples stored for a shorter period. Inactivation during storage is probably a continuous process, a fact that may interfere with our results, but would lead to false low levels of bFGF and rather attenuate the results.

Our results clearly demonstrate the positive correlation of serum bFGF to tumour stage and nuclear grade

Table 2
Serum bFGF in relation to tumour vein invasion in patiens with renal cell carcinoma

Vein invasion	No.	Not detectable (<1.0 pg/ml)	bFGF (pg/ml)	⩾5 pg/ml	P value[a]
		No. (%)	Median (range)		
No invasion	125	44 (35)	2.7 (<1–38.0)	45	
Vena renalis	49	7 (14)	3.9 (<1–70.9)	23	0.007
Vena cava	26	3 (12)	4.9 (<1–25.4)	12	

[a] Mann–Whitney test.

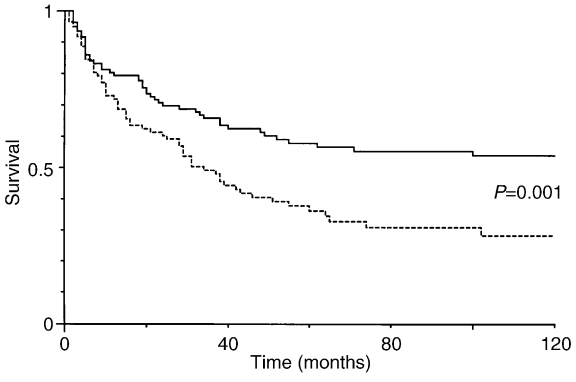

Fig. 2. Cause-specific survival of patients with renal cell carcinoma according to the initial serum basic fibroblast growth factor (bFGF) level. 107 patients had bFGF ⩽3.0 pg/ml (—), and 99 had levels >3.0 pg/ml (- - - - -).

Table 3
Univariate analysis of prognostic factors in patients with renal cell carcinoma

Prognostic factor	Odds ratio (95% CI lower–upper)
Gender	
Male	1.0
Female	0.92 (0.63–1.36)
Age (years)	
⩽66	1.0
>66	1.02 (0.70–1.49)
Tumour stage	
I–II	1.0
III–IV	12.87 (7.25–22.85)
Grade	
1–2	1.0
3–4	6.97 (3.23–15.03)
Vein invasion	
Absent	1.0
Present	3.63 (2.44–5.41)
bFGF (pg/ml)	
⩽3.0	1.0
>3.0	1.80 (1.23–2.65)

CI, confidence interval.

Table 4
Multivariate analysis of prognostic factors in patients with renal cell carcinoma (vein invasion and bFGF not prognostic)

Prognostic factor	Odds ratio (95% CI lower–upper)
Tumour Stage	
I–II	1.0
III–IV	10.78 (5.55–20.99)
Grade	
1–2	1.0
3–4	2.36 (1.03–5.41)
Vein invasion	
Absent	1.0
Present	0.79 (0.51–1.22)
bFGF (pg/ml)	
⩽3.0	1.0
>3.0	1.17 (0.74–1.68)

CI, confidence interval.

in patients with renal cell carcinoma. These observations are in accordance with earlier reports [17,20]. Although our reference group was limited, the results indicate a difference between serum levels of bFGF in patients with renal cell carcinoma compared with benign renal cysts. This observation confirms earlier findings of Ii and associates [18] and Dosquet and associates [20], even though sera from healthy subjects were used as controls in those studies.

Using immunohistochemical staining, Nanus and associates [11] demonstrated bFGF expression in blood vessel walls of normal kidney, in the extracellular matrix surrounding renal cancer cells and in renal cancer cell cytoplasm. The expression of bFGF in vessel walls and extracellular matrix occurred in 98 and 87% of the 62 tumours, respectively. In contrast, only 16% of the tumours stained positively in the cytoplasm. Fujimoto and associates [17] analysed bFGF selectively in the renal vein of patients with renal cell carcinoma. In 2 patients, they actually demonstrated increased levels of bFGF in the affected vein, even though the level of bFGF in the peripheral blood was normal. Furthermore, they analysed serum bFGF in 7 patients before and after nephrectomy, and found that the level was normalised in 5 of these patients 2 weeks after surgery. These observations are in favour of the hypothesis that the source of bFGF is the renal tumours.

This study also shows that serum bFGF levels are higher in patients with tumour thrombus in the vein system. This observation is in accordance with the work of Fujimoto and associates [16,17]. They report that in patients with tumours without vein invasion, only 32% had elevated serum bFGF, compared with 88 and 80% for patients with tumours invading the renal vein or the caval vein, respectively. In renal cell carcinoma, there are conflicting observations concerning the prognostic impact of microvascular density [7,8]. The expression of bFGF assayed by means of immunohistochemical staining indicated poor survival, but was not identified as an independent prognostic factor [11]. Nor was the serum level of bFGF an independent prognostic factor when analysed by Dosquet and associates [20]. Our results confirm these observations, but further investigations are needed to elucidate the role of angiogenic factors and their impact on prognosis.

New treatments are needed for patients with advanced renal cell carcinoma, and anti-angiogenic therapy may be of value. It has been shown that interferon-α downregulated the expression of bFGF in renal cell carcinoma in cell cultures [30]. Similar results were found when nude mice with transplanted bladder carcinoma were treated with interferon-α [31] and Vermeulen and associates [32] found, in a limited number of patients with lung metastases of renal cell carcinoma, that the serum bFGF level predicted the response to interferon therapy. The expression of bFGF might possibly indicate the sensitivity to anti-angiogenic therapy.

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