Basic Fibroblast Growth Factor as a Candidate Tumor Marker for Renal Cell Carcinoma

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The present investigation was conducted to determine serum levels of basic fibroblast growth factor (FGF) by enzyme immunoassay in patients with various urogenital tumors. Renal cell carcinoma had a higher tendency (28 of 52, 53.8%) toward increased serum levels of basic FGF than any of the other urogenital tumors, and increased serum basic FGF was detected more frequently in patients with advanced renal cell carcinoma. Analysis of histological pattern indicated that renal cell carcinoma with a solid or tubular component is more likely to produce basic FGF. However, no significant difference was seen between the percentage of clear cell type tumor patients with increased serum basic FGF (50.0%) and the percentage of granular cell type tumor patients with increased serum basic FGF (66.7%). Five of 8 patients with renal cell carcinoma who underwent selective renal venous sampling before nephrectomy showed increased serum basic FGF in the renal vein from the affected kidney. After resection of the affected kidney to remove the cancer, serum basic FGF disappeared within 2 weeks. However, residual huge tumor or postoperative disease prolonged the increased levels of basic FGF in 2 patients, indicating that basic FGF is produced from and secreted by tumor tissue itself. These findings suggest that serum basic FGF can be useful in the diagnosis, and in evaluating the prognosis, of patients with renal cell carcinoma.

Key words: Basic fibroblast growth factor — Enzyme immunoassay — Renal cell carcinoma — Tumor marker

Basic fibroblast growth factor (FGF) is an extracellular component of many organs and tissues, and has been well characterized. A variety of biological actions have been demonstrated, including promotion of growth, differentiation, cell survival and angiogenesis, an important stage of tumor growth and development.[1-3] In this study, we focused on the mechanism of tumor angiogenesis and the function of angiogenic growth factor in relation to the behavior of malignant tumors such as renal cell carcinoma. Angiography or histopathological diagnosis shows significant tumor vascularization in most renal cell carcinomas. We suggest that hypervascularity in renal cell carcinoma is closely related to angiogenic growth factor as well as to basic FGF.

We previously reported that increased serum levels of basic FGF are often observed in patients with advanced renal cell carcinoma, and suggested that this substance could be a marker for renal cell carcinoma.[4] Recently Yamanaka et al.,[5] using Northern blot analysis, reported that acidic FGF and basic FGF are overexpressed in a large proportion of patients with pancreatic cancer. Watanabe et al.[6] suggested that basic FGF can be useful in evaluating the prognosis of breast cancer. However,

few studies have investigated the source of increased serum basic FGF in patients with a variety of tumors.

We examined the serum levels of basic FGF using a two-site enzyme immunoassay in patients with a variety of urogenital tumors to determine whether increased serum levels of basic FGF are specific for hypervascular tumors such as renal cell carcinoma. To determine the source of serum basic FGF in renal cell carcinoma, we sampled serum from the renal veins bilaterally and determined the serum levels of basic FGF after nephrectomy.

MATERIALS AND METHODS

Antibodies The characteristics of these antibodies have been described in detail.[7] Three monoclonal antibodies (MAb) against recombinant human basic FGF were used in this experiment. MAb52 and MAb98, employed as solid-phase antibodies for coating of microtiter plates, identified various epitopes of human basic FGF. The Fab' fragment of MAb3H3 was labeled with horseradish peroxidase. These antibodies displayed no cross-reactivity with acidic FGF or hst-1 gene product.

Serum and urine Sera were obtained from 52 patients with renal cell carcinoma, 4 with renal pelvic and ureteral cancer, 26 with bladder cancer, 12 with prostate cancer,

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and 12 with testicular tumor. Blood was collected from patients before breakfast and centrifuged for 10 min at 1100g. We tested 18 urine samples from patients with renal cell carcinoma, 7 from patients with bladder cancer, and 4 from patients with prostate cancer. The urine was also collected by centrifugation for 10 min at 650g. Serum and urine samples were stored at −80°C until assayed.

Venous blood was also sampled at the time of angiographic examination or during surgery in 8 patients with renal cell carcinoma. Blood was collected from the renal veins bilaterally and from the vena cava both above and below the site of renal vein bifurcation.

Serum basic FGF was measured before and after nephrectomy in 7 patients with renal cell carcinoma.
Two-site enzyme immunoassay Serum basic FGF was measured by two-site sandwich enzyme immunoassay (EIA), as we described previously.[4] In brief, the limit of detection of basic FGF in serum was 30 pg/ml. A mixture of two solid-phase antibodies, MAb52 and MAb98, was adjusted to $10 \mu g/ml$ in 0.1 M carbonate buffer (pH 9.6). A volume of $100 \mu l$ of the mixture was incubated in the wells of a 96-well microtiter plate at 4°C overnight. The plate was washed several times in PBS (0.02 M phosphate buffer, pH 7.2, with 0.15 M NaCl), then $300 \mu l$ of Buffer A (PBS containing 25% Block Ace; Snow Brand Milk Products Co., Sapporo) was added to each well and the plate was incubated at 4°C overnight. It was washed in PBS, and $100 \mu l$ of sample mixture, 3-fold diluted in an equal volume of Buffer B (Buffer A containing $10 \mu g/ml$ heparin) and an equal volume of Buffer C (Buffer A containing 1.5 M NaCl, $100 \mu g/ml$ heparin and $30 \mu g/ml$ mouse IgG) was applied. Incubation was continued at 4°C for 24 h. Then the plate was washed in PBS several times, and $100 \mu l$ of MAb3H3 labeled with horseradish peroxidase, 200-fold diluted in Buffer A with 10 $\mu g/ml$ of mouse IgG, was incubated in each well at 25°C for 2 h. The bound peroxidase activity was counted with o-phenylenediamine as a substrate. In this immunoassay system, the detection limit of serum or urine basic FGF was 30 pg/ml.

Table I. Serum and Urine Basic FGF Detected by Enzyme Immunoassay in Human Urogenital Tumors

Tumor	No. of positive patients/No. of total patients (%)	
	Serum	Urine
Renal cell carcinoma	28/52 (53.8)*	0/18
Urothelial cancer	8/30 (26.7)	1/8 (12.5)
Testicular tumor	0/12	not examined
Prostatic cancer	2/7 (28.6)	0/4

* $P < 0.05$, renal cell carcinoma versus urothelial cancer. $P < 0.001$, renal cell carcinoma versus testicular tumor.

Others None of the patients had received chemotherapy or radiation therapy before samples were collected. All resected tumor specimens were diagnosed pathologically. In patients with renal cell carcinoma, tumor grade, stage, subtype, and histological pattern were diagnosed pathologically according to the General Rules for Clinical and Pathological Studies on Renal Cell Carcinoma[8] based on the TNM Classification of Malignant Tumors by UICC,[9] with minor modifications. Maximum diameter was measured for 47 fresh tumor tissues before fixation.

RESULTS

Basic FGF in urogenital tumors Table I summarizes the serum or urine levels of basic FGF in various urogenital cancers. Of 52 patients with renal cell carcinoma, 28 (53.8%) showed increased serum levels of basic FGF, but none of the 18 urine samples from those patients revealed an increase. The incidence of increased serum basic FGF of renal cell carcinoma was significantly higher than that of urothelial cancer or testicular tumor.
Serum basic FGF in renal cell carcinoma All specimens from renal cell carcinoma patients were staged and graded histopathologically to assess the correlation between serum levels of basic FGF and histopathological parameters. One of 2 patients with pT1 showed increased serum basic FGF (32 pg/ml). Ten of 27 patients (37.0%) with pT2 showed increased serum levels of basic FGF (30–353, 98.7±103.9 pg/ml), while 15 of 20 patients (75.0%) with pT3 (36–540, 160.9±134.6 pg/ml) and 2 of 3 (66.7%) with pT4 (417 and 492 pg/ml) also showed increased serum levels of basic FGF (Table II).

Increased basic FGF was more frequently detected in sera of patients in whom the tumor had invaded the renal vein or vena cava. In patients with renal cell carcinoma without venous invasion (pV0), only 10 of 31 patients (32.3%) showed increased serum levels of basic FGF (30–353, 104.6±103.9 pg/ml). Basic FGF was detected in 14 of 16 patients (87.5%) with tumor invasion of renal veins (43–280, 159.9±143.9 pg/ml) and 4 of 5 patients (80.0%) with tumor invasion of the vena cava (41–540, 264.0±216.7 pg/ml) (Table II).

Increased serum levels of basic FGF were also found in patients with higher-grade tumors. Five of 20 patients (25.0%) with grade 1 renal cell carcinoma showed increased serum basic FGF (57–320, 119.6±112.3 pg/ml), as did 16 of 23 patients (69.6%) with grade 2 (30–353, 130.3±130.5 pg/ml), and 7 of 9 patients (77.8%) with grade 3 (41–540, 236.9±187.9 pg/ml) (Table II).

Renal cell carcinoma is usually comprised of three cellular subtypes, i.e., clear, granular and pleomorphic. We observed no significant difference between the percentage of patients with clear cell-type renal cell carcinoma who showed increased serum basic FGF and the percen-

Table II. Serum Basic FGF and Histopathological Findings in Patients with Renal Cell Carcinoma

	No. of positive patients/No. of total patients (%)	Mean basic FGF level of positive patients (pg/ml)
Histopathological stage		
pT1+pT2	11/29 (37.9)	92.6±100.6
pT3	15/20 (75.0)	160.9±134.6
pT4	2/3 (66.7)	454.5±53.0
Venous invasion		
pV0	10/31 (32.3)*	104.6±103.9
pV1	14/16 (87.5)*	159.9±143.9
pV2	4/5 (80.0)*	264.0±216.7
Histopathological		
G1	5/20 (25.0)**	119.6±112.3
G2	16/23 (69.6)**	130.3±130.5
G3	7/9 (77.8)**	236.9±187.9
Total	28/52 (53.8)	155.0±146.6

* $P<0.005$ (χ^2 test). ** $P<0.001$ (χ^2 test).

tage of patients with granular cell-type carcinoma who exhibited this increase. Among 52 patients with renal cell carcinoma, 18 of 36 patients (50.0%) with clear cell type and 4 of 6 (66.7%) with granular cell type were found to have increased basic FGF in the sera (30–492, 116.4±120.0 pg/ml; 41–540, 237.8±214.0 pg/ml). One pleomorphic type showed increased serum basic FGF (417 pg/ml). Five of the remaining patients in whom a mixture of clear cell and granular cell subtypes was identified were also found to have elevated serum basic FGF (43–353, 175.6±135.5 pg/ml).

Histological patterns were determined according to the following morphological features: solid, tubular, alveolar, cystic and papillary patterns. Table III illustrates the predominant patterns. As shown in the table, renal cell carcinoma was often characterized by a combination of several patterns. Solid and tubular patterns were more frequently associated with increased serum levels of basic FGF than were the cystic or alveolar patterns.

Forty-five resected renal tumors were divided into two groups based on the maximum diameter of the primary tumor: ≧5 cm and <5 cm. Seventeen of 29 (58.6%) renal cell carcinomas with diameter ≧5 cm showed increased serum basic FGF, and 6 of 16 (37.5%) with diameter <5 cm showed increased serum levels of basic FGF, though there was not a significant difference between these two groups.

Selective renal venous sampling In 7 patients who underwent preoperative angiography, venous blood was sampled at either four or two sites, including the bilateral renal veins and the inferior vena cava above and below the site of renal vein bifurcation. Two patients with peripheral serum basic FGF <30 pg/ml had no increase in serum level of basic FGF in the renal vein. Three patients showed increased basic FGF in sera obtained from the

Table III. Serum Basic FGF and Histological Patterns in Patients with Renal Cell Carcinoma

Histological pattern	No. of positive patients/No. of total patients (%)
Cystic+Tubular	0/2 (0)
Cystic+Alveolar	1/2 (50.0)
Alveolar	10/25 (40.0)
Alveolar+Tubular	2/5 (40.0)
Papillary	2/4 (50.0)
Tubular	4/4 (100.0)
Solid	7/7 (100.0)
Solid+Alveolar	2/3 (66.7)

affected kidney as well as from the peripheral blood. In one patient with tumor thrombus in the vena cava and the renal veins, venous sampling from the bilateral renal veins was not possible. This patient showed a high level of serum basic FGF in the vena cava above the site of bifurcation.

Basic FGF after nephrectomy We also measured serum levels of basic FGF after nephrectomy in 7 patients with renal cell carcinoma whose serum levels of basic FGF were elevated prior to the operation. Five patients showed decreases of <30 pg/ml of basic FGF. Serum levels of basic FGF after nephrectomy did not decrease at all in one patient who had a huge residual tumor in the vena cava with lymph node metastases and in another who had protracted serious postoperative ileus.

DISCUSSION

The detection of renal cell carcinoma is increasing because of the development and widespread use of mass

screening for a variety of other diseases. However, there is no radical or available treatment for renal cell carcinoma except surgical resection of this malignant tumor, so that early detection and treatment are crucial. One approach is to develop a tumor marker specific for renal cell carcinoma based on its biological features.

Tumor growth and development are thought to be spurred by angiogenic mitogen.[1, 2] Angiogenesis is more likely to be relevant to the growth of hypervascular tumors. As we reported in a previous study, basic FGF is frequently detected in patients with advanced renal cell carcinoma and may offer a novel marker for renal cell carcinoma.[4] In this study, we examined serum and urine levels of basic FGF in patients with renal cell carcinoma and other urogenital tumors to clarify our earlier findings.

The present observations indicate that an increase in serum basic FGF was more commonly detected in patients with renal cell carcinoma than in patients with other urogenital tumors, suggesting that basic FGF is relatively specific for renal cell carcinoma, and that it contributes to the angiogenesis of this hypervascular tumor.

Nguyen et al.[10, 11] reported that an abundance of basic FGF was detected in patients with many types of cancers including bladder cancer. In the urine assay, no basic FGF was detected in patients with renal cell carcinoma, prostate cancer, renal pelvic cancer or ureteral cancer, with the exception of 1 of the 7 patients with bladder cancer. However, hematuria or inflammation of the uroepithelium may have brought about this result. Soutter et al. suggested that basic FGF in the urine of cancer patients is generated by the tumor itself.[12]

The present findings confirm that advanced renal cell carcinoma of a higher stage than pT3 or pV1 or a higher grade than G2 is characterized by increased serum levels of basic FGF. Renal cell carcinomas, including those with a solid or tubular component, showed increased serum levels of basic FGF. This confirms the clinical observation that high-grade solid tumors are the most aggressive and have the poorest prognosis. No significant difference was noted however, between the percentage of patients with clear cell-type renal cell carcinoma who showed increased serum basic FGF and the percentage of granular cell-type patients who showed this increase. Thus, increase of serum basic FGF does not appear to depend on the cell type. Basic FGF may be produced and secreted from tumor tissue with pathologically high-grade malignancy. However, there was no significant difference in the percentage of carcinomas with elevated basic FGF between the group with maximum tumor diameter ≥ 5 cm and the group with maximum diameter < 5 cm. These results suggest that some small renal cell carcinomas are already aggressive and have reached a high degree of malignancy due to their rapid growth, early invasion and metastases, showing high levels of basic FGF. Grade 3 or solid component small renal cell carcinomas seem to secrete more basic FGF than do high-stage tumors with low malignancy, such as grade 2 or cystic pattern.

We also evaluated the serum levels of basic FGF at each site of the bilateral renal veins and venae cavae in order to detect basic FGF in patients with no increase of basic FGF in peripheral blood. Increased basic FGF could be detected even in early carcinoma with low-grade malignancy in selectively collected venous samples. Two patients in this study actually showed increased serum basic FGF in the affected renal vein, even though they failed to show an increase in serum basic FGF in the peripheral blood.

Increased serum levels of basic FGF returned to normal within two weeks after resection of the tumor by nephrectomy. However, high levels of basic FGF were protracted in patients with residual tumor mass or postoperative complications, such as serious ileus, wound infection or subcutaneous abscess. This suggests that basic FGF plays an important role in wound healing.

We also investigated the localization of basic FGF in eight renal cell carcinomas by immunohistochemistry using MAb3H3. One renal cell carcinoma showed intensive staining of tumor cells corresponding to the nuclei, while the others showed negative or weak staining of tumor cells. However, basic FGF was clearly and widely detected in endothelial cells, macrophages, and extracellular matrix surrounding the tumor cells (unpublished data). The immunohistochemical results support the view that basic FGF is secreted from tumor tissue itself; predominantly from endothelial cells or extracellular matrix and partially from tumor cells themselves. It is also suggested that tumor angiogenesis is not only controlled by the presence of basic FGF, but also is mediated by other angiogenic inducers and inhibitors.

In conclusion, basic FGF is closely involved in the development and growth of hypervascular tumors such as renal cell carcinoma. Basic FGF is a prime candidate for a tumor marker with a high specificity for this malignant disease.

ACKNOWLEDGMENTS

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