Increased Levels of Fibroblast Growth Factor-like Activity in Urine from Patients with Bladder or Kidney Cancer

Gerald W. Chodak,2 Verne Hospelhorn, Sheila M. Judge, Ruth Mayforth, Hartmut Koeppen, and Joachim Sasse

ABSTRACT

Growth factor activity was partially purified from human renal tumors and a human bladder cancer cell line by heparin-Sepharose chromatography. This activity stimulated bovine capillary endothelial cell proliferation and DNA synthesis in BALB/c 3T3 cells. Partially purified growth factor preparations from these tumors contained a protein with an approximate molecular weight of 17,000 which was recognized by a polyclonal antiserum raised against a peptide fragment of basic fibroblast growth factor (FGF). This growth factor activity appears to be related to basic fibroblast growth factor.

Measurement of FGF-like activity in 50 urine samples from 32 adult males showed that 55% (6 of 11) of the urine samples from patients with bladder cancer and 100% (7 of 7) of the urine samples from patients with kidney cancer contained activity equivalent to more than 20 ng of basic FGF/h of urine production. In contrast, only 6% (2 of 32) of the urine samples from controls, patients with a benign disease, or patients with a history of bladder or kidney cancer contained this level of growth factor activity. These results suggest that patients with bladder or kidney cancer release an FGF-like factor into urine which may be used as a marker for these tumors.

INTRODUCTION

The development of new capillaries plays a fundamental role in the growth of solid tumors (1). Because tumor-induced angiogenesis is caused by diffusible factors (2), bodily fluids surrounding tumors may contain increased levels of angiogenic activity. For example, significantly more angiogenic activity has been detected in cerebrospinal fluid obtained from patients with brain tumors (3), in aqueous humor from patients with eye tumors (4), and in urine from patients with bladder cancer (5) in comparison to healthy controls and patients with benign disease.

One of the factors that stimulates angiogenesis is basic FGF3 (6). This protein belongs to a family of proteins that have been identified in a wide variety of normal and malignant tissues (7). Basic FGF is a 146 amino acid, single chain protein (8) that is cationic, binds to heparin with high affinity, and stimulates the growth of capillary endothelial cells (7).

For the last few years, we have studied the relationship between carcinoma of the bladder and the angiogenic growth factor content in urine (9, 10). Bladder cancer is a recurring disease, and detection usually requires invasive tests which are painful and uncomfortable for the patient. A non-invasive test for bladder tumors would be useful for detecting early cancers, monitoring the effectiveness of therapy, and detecting recurrence.

Several lines of evidence suggest that patients with bladder or kidney cancer release a factor into urine that may be used as a marker for these tumors. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Preparation of Anti-FGF Polyclonal Antiserum

An 11-amino acid fragment of the carboxy-terminal domain of human basic FGF (Asp-Glu-Val-Asp-Val-Lys-Val-Lys-Ser-Ser-Cys) was synthesized as a solid-phase peptide using an automated Applied Biosystems 430-A peptide synthesizer (12). The synthetic peptide was conjugated to KLH (Boehringer Mannheim, Indianapolis, IN) according to previously described methods (14) using maleimidobenzoyl-N-hydroxysuccinimide ester as a cross-linking agent. The peptide-carrier conjugate was dialyzed against PBS and stored frozen at −20°C.

Polyclonal antibodies were produced by giving 8-lb male New Zealand White rabbits injections at multiple dorsal i.d. sites of 0.5 mg of KLH-peptide conjugate emulsified in complete Freund's adjuvant and boosted at 4- to 6-wk intervals.

Collection and Storage of Urine Samples

Voided urine was collected and pooled from either healthy males or male patients with histologically proven Stage B transitional cell carcinoma of the bladder. The urine was centrifuged at 627 × g for 10 min and then stored at −70°C. Individual urine samples were arbitrarily collected from hospitalized male patients, healthy male volunteers, and factors may be useful as a marker for this disease. In this paper, we show that men with bladder or renal tumors can be distinguished from normal men by the presence of higher levels of a heparin-binding, angiogenic-like growth factor in their urine which is similar to basic fibroblast growth factor.

MATERIALS AND METHODS

Preparation of Tumor Extracts

Fresh tumor tissue was obtained through the Department of Pathology at the University of Chicago from patients undergoing a radical nephrectomy for adenocarcinoma of the kidney. Tissue was also obtained from physicians at the University of Iowa and the University of Texas Medical Center. Samples were frozen immediately and stored at −70°C until use. Crude tumor extracts were prepared by thawing the tumors, homogenizing them in a blender, and then treating the homogenate with 2 mg/ml of collagenase in 10% phosphate-buffered saline for 3 h at 37°C. Tumor digests were filtered through 110-μm nylon mesh and sterile filtered through 0.45-μm filters, and the extracts were stored at −70°C.

Establishment of Human Bladder Tumor Cell Line (HB-1)

Tumor tissue fragments were obtained from a male patient undergoing a transurethral resection of a bladder tumor. The fragments were cut into 1-mm3 pieces and placed in RPMI 1640 (Gibco Laboratories, Grand Island, NY). The pieces were placed in two flasks (25 and 75 cm2) in RPMI 1640/10% FBS (Gibco)/10% NCTC 109 (Gibco)/1% penicillin and streptomycin and incubated at 37°C. Outgrowths from the explants were cloned by limiting dilution to remove contaminating normal fibroblasts. Subconfluent monolayers of cloned tumor cells were trypsinized, harvested, and resuspended in RPMI 1640/10% FBS. Athymic nude BALB/c mice (Frederick Cancer Research Institute, Bethesda, MD) were given injections s.c. of 1 to 2 x 106 cells. When the diameter of the tumor reached 2 cm (approximately 10 wk after the cells were injected), the tumor was removed for histological examination and readaptation to tissue culture.

Preparation of Antipeptide Antibodies

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: FGF, fibroblast growth factor; FBS, fetal bovine serum; KLH, keyhole limpet hemocyanin; DMEM, Dulbecco's modified essential medium; CRF, crude renal factor; BCE, basic capillary endothelial; TGF-α, transforming growth factor-α; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; PBS, phosphate-buffered saline.

Received 4/23/87; revised 10/23/87, 12/28/87; accepted 1/20/88.

The authors thank the following institutions for their support and assistance: The Cancer Research Endowment Fund of the University of Chicago, the Cancer Research Foundation, and a gift from the Johnson & Johnson Corporation. To whom requests for reprints should be addressed, at Box 403, the University of Chicago Medical Center, Chicago, Illinois 60637. This is article 48, 2083-2088, April 15, 1988.
male patients visiting the outpatient Urology Clinic. Patients were selected from a single ward in the hospital which is shared by General Surgery and Urology. To determine the collection period for each sample, the time of last urination was recorded, and all subsequent samples were collected for a minimum period of 6 h. At the end of the collection period, the time of the patient’s last urination was recorded, and the last specimen was included in the total collection. Each specimen was kept on ice during collection and then centrifuged at 627 × g for 30 min to remove cells and debris. The samples were stored individually at −70°C until use. Grossly bloody specimens were excluded from analysis (these represented less than 5% of the samples).

The age, diagnosis, date of collection, current medications, stage of disease (for cancer patients), total volume per sample, and duration of sample collection were recorded for each patient. The stage of each tumor was assessed by radiological methods and/or by histopathological assessment following biopsy or surgical excision.

In some cases, multiple samples were tested from the same patient to determine interassay variability. To avoid biasing the results, replicate samples from the same patient were averaged, and the average value was combined with the results from other patients in the same diagnostic group.

Bio-Rex 70 Cation Exchange Chromatography

Crude renal tumor extract was applied to a 1- × 10-cm column containing Bio-Rex 70 (200 to 400 mesh; Bio-Rad) equilibrated with 0.1 M NaCl/0.01 M Tris-HCl (pH 7.0). Growth factor was eluted with a 200-ml gradient of 0.1 to 1.0 M NaCl in Tris buffer at a flow rate of 30 ml/hr as previously described (15). Ten μl of every third fraction were tested in the 3T3 cell assay.

Heparin-Sepharose Chromatography

Tumor Extracts. Partially purified extracts from human renal cell carcinoma or the human bladder cancer cell line, HB-1, were applied to 8-ml heparin-Sepharose columns (10 × 1 cm; Pharmacia) in 0.01 M Tris, pH 7.0, at 4°C. The column was washed with 0.01 M Tris/0.1 M NaCl (pH 7.0). FGF-like growth factors were eluted by either of two methods: initially, a 0.1 to 3.0 M NaCl gradient in 0.01 M Tris (pH 7.0) was used to elute the column. Once we established that tumor-derived, heparin-binding growth factor activity was consistently eluted by 1.2 to 1.4 M NaCl, subsequent columns were eluted step-wise with 0.6 M NaCl, followed by 1.5 M NaCl/0.01 M Tris (pH 7.0).

Pooled Urine Samples. To determine if urine contained heparin-binding growth factor activity, urine samples were thawed, pooled, and then mixed overnight with 5 to 8 ml of heparin-Sepharose at 4°C on a shaker. After decanting the urine, the gel was poured into a 1- × 10-cm column and washed with 30 ml of 0.6 M NaCl/0.01 M Tris (pH 7.0). Growth factor activity was eluted with a 0.1 to 3.0 M NaCl gradient in 0.01 M Tris (pH 7.0). One-ml fractions were collected and stored at −70°C until analyzed.

Analysis of Growth Factor Activity

3T3 Cell Assay. Growth factor activity in column eluates was assessed by measuring the incorporation of [3H]thymidine by quiescent monolayer cultures of BALB/c 3T3 cells as described previously (16). Each plate included control cultures that were fed DMEM containing 4.5 g of glucose/liter, 50 units/ml of penicillin, 50 μg/ml of streptomycin, 1% FCS, and 10% calf serum. The column eluates were tested in duplicate by adding 5 or 10 μl to each well. The incorporation of [3H]thymidine in the control sample was subtracted from the value for each test sample. One unit of growth factor activity was defined as one-half the amount of [3H]thymidine incorporation obtained when 50 μl of calf serum were added to control cultures.

Capillary Endothelial Cell Proliferation Assay. Bovine capillary endothelial cells (clone 76-23, kindly provided by J. Folkman) were grown in gelatin-coated dishes as previously described (17) in DMEM/10% calf serum/penicillin (50 units/ml)/streptomycin (50 μg/ml)/1% FCS/CRF (1 μl/ml) (18). CRF has been shown to contain acidic FGF which is a mitogen for BCE cells (18). Confluent cultures were passaged each week by detachting the cells with 0.25% trypsin-0.01% EDTA and seeding the cells in growth medium. Stimulation of cell proliferation was measured by seeding 10,000 to 15,000 capillary endothelial cells/well in a 24-well plate containing 0.5 ml/well of the growth medium without CRF. The cells were incubated at 37°C overnight in 10% CO2, and the following day the media were replaced with fresh media also lacking CRF. Test samples (25 to 75 μl) were added to duplicate wells and incubated at 37°C for 72 h. The media were then removed, the cells were trypsinized, and the number of cells in each sample was determined by counting aliquots of the cell suspension in a Model ZF Coulter Counter.

Each assay included a negative control (cells plus culture medium with no added sample) and a positive control (cells plus culture medium plus 25 μl of CRF). The results of an experiment were accepted as valid only if CRF induced at least a 50% increase in cell number compared to the negative control.

Western Blot Analysis of Partially Purified Growth Factor

Growth factor activity from bladder and kidney tumor extracts was partially purified by heparin-affinity chromatography. Samples were electrophoresed on polyacrylamide gels according to standard procedures (19). The gels were overlayed with 0.22-μm-pore nitrocellulose membranes (Schleicher and Schuell, Keene, NH), and protein transfers were performed at 25 V overnight at 4°C. Prior to immunoblotting, the membranes were incubated with 3% nonfat dried milk in PBS for 30 min to prevent nonspecific binding. Reagents were added as previously described using anti-FGF (peptide) antibodies (20). All antibody dilutions were performed in PBS containing 1% milk and 0.1% Tween-20.

Measurement of Heparin-binding Growth Factor Activity in Individual Human Urine Samples

Each urine sample was thawed and centrifuged at 855 × g for 20 min at 4°C, and the supernatant was adjusted to 50 mM Tris (pH 7.0). Each sample was mixed with 0.3 g of heparin-Sepharose on an orbital shaker overnight at 4°C. The next morning, the mixture was centrifuged at 213 × g for 10 min, and the urine was decanted. The heparin-Sepharose was transferred to 1- × 5-cm columns (Bio-Rad) and washed with 30 ml of 0.6 M NaCl/0.01 M Tris (pH 7.0), and growth factor was eluted with 2.2 ml of 3.0 M NaCl/0.01 M Tris (pH 7.0). In previous studies, we found that this volume was sufficient to elute 90% of growth factor activity from the column. Two-ml aliquots of the 3.0 M salt eluate were transferred to microcentrifuge tubes (Amicon, Danvers, MA) and centrifuged for 1 h at 4°C. The samples were desalted twice by adding 2 ml of 5 mM Tris/0.01 M NaCl to the tubes and centrifuging for 1 h. The final volume of the desalted, concentrated samples was adjusted to 170 μl. Fifty μl of each sample were tested in triplicate in the 3T3 cell assay.

RESULTS

Characterization of Human Bladder Tumor Cell Line, HB-1.

To determine if human bladder cancer contains bFGF-like growth factor activity, a human cell line was established. HB-1 cells grown in nude mice produced tumors that were histologically identical to the original tumor. Both the histology of the tumor and the morphology of the tumor cells as determined by electron microscopy were consistent with transitional cell carcinoma (data not shown).

To establish the human origin of the HB-1 cell line, we analyzed the reaction between species-specific monoclonal antibodies and the HB-1 cells by fluorescence-activated cell sorting. The HB-1 cells reacted strongly with the monoclonal antibody BBM.1, which recognizes human, but not mouse, β2-microglobulin (21), and with antibody W6/32, which recognizes a common HLA-A,B,C framework determinant (22). These findings indicate that HB-1 cells are of human origin. The tumor cells also reacted weakly with monoclonal antibody

---

*Unpublished data.*
9A7, an antibody which is thought to recognize an antigen present on many human transitional carcinomas (23).

Growth Factor Activity in HB-1 Human Bladder Carcinoma Cells and Adenocarcinoma of the Kidney. Previous studies have shown that angiogenic fibroblast growth factors produced by tumors can be partially purified by heparin-affinity chromatography (7). To isolate growth factor activity from HB-1 cells, crude extracts (6 mg of protein/ml) of these cells were applied to a heparin-Sepharose affinity column, and protein was eluted stepwise with increasing concentrations of NaCl. The eluates were tested for growth factor activity in the 3T3 and BCE cell assays. Although many growth factors stimulate 3T3 cells, only angiogenic factors are known to stimulate BCE cells. The 1.5 m NaCl eluate contained both 3T3 cell and BCE cell growth factor activity. The 0.6 m eluate stimulated 3T3 cells but not BCE cells.

Because the protein content of crude kidney tumor extract was high (30 mg of protein/ml), it was necessary to partially purify growth factor activity by cation-exchange chromatography on Bio-Rex 70 prior to heparin-Sepharose chromatography (7). The 3T3 and BCE cell growth factor activities coeluted from Bio-Rex 70 with 0.5 to 0.6 m NaCl in agreement with previous findings (7). Growth factor activity was further purified by chromatography on heparin-Sepharose using a 0.1 to 3.0 m NaCl gradient. The 1.2 to 1.4 m NaCl eluate contained growth factor activity that stimulated both 3T3 and BCE cells (Fig. 1).

Western Blot Analysis of FGF-related Proteins in Partially Purified Preparations from MB-1 Cells and Renal Tumors. To characterize the heparin-binding growth factor extracted from bladder and kidney tumors, we determined the immunoreactivity of these factors with anti-bFGF antiserum. The specificity of the anti-bFGF antiserum was demonstrated by Western blot analysis of recombinant bovine basic FGF (supplied by Amgen Corp.) and CRF. Both tumor extracts and bFGF contained a protein with approximate molecular weight of 17,000 which strongly reacted with the anti-FGF (peptide) antiserum (Fig. 2A). This reaction could be blocked by prior incubation of the antiserum with the FGF peptide that was used as the original antigen. The antiserum did not recognize acidic FGF present in CRF. Thus, the antiserum is specific for basic FGF.

Western blot analysis of growth factor activity partially purified by heparin-affinity chromatography from HB-1 bladder tumor cells and human kidney tumors showed that both of these preparations contained a protein with an approximate molecular weight of 17,000 that binds anti-bFGF (peptide) antibody (Fig. 2B).

Growth Factor Activity in Human Urine. We previously postulated that angiogenic factors produced by bladder tumors were released into urine (8). To determine if urine contained a heparin-binding growth factor similar to the bFGF-like factor found in HB-1 bladder cells and kidney tumors, we analyzed human urine samples. Ten liter of urine that were pooled from eight patients with bladder cancer were tested for growth factor activity after gradient elution from heparin-Sepharose. The elution profile of urine shown that 3T3 cell growth factor activity eluted at 1.2 to 1.5 m NaCl similar to the tumor extracts. Urine also contained a second peak of 3T3 cell growth factor activity that was eluted by 1.8 to 2.2 m salt (Fig. 3).
FIBROBLAST GROWTH FACTOR-LIKE ACTIVITY IN HUMAN URINE

To determine whether the amount of heparin-binding growth factor activity in urine was affected by the presence or absence of a tumor, we compared the amount of 3T3 and BCE cell growth factor activity in 4 liters of urine that were pooled, concentrated 10-fold, and applied to a heparin-Sepharose column. Growth factor was eluted with a 0.1 to 3.0 M NaCl gradient. Fractions were pooled sequentially in groups of three, dialyzed, and tested for 3T3 cell growth factor activity.

Table 1 Heparin-binding growth factor activity in pooled urine

<table>
<thead>
<tr>
<th>Urine source</th>
<th>3T3 cells*</th>
<th>BCE cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder cancer patients</td>
<td>53</td>
<td>192</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>13</td>
<td>24</td>
</tr>
</tbody>
</table>

* One unit of 3T3 cell growth factor activity is defined as one-half the stimulation of [3H]thymidine incorporation obtained by treatment of the cells with 0.05 ml of cell serum.

Table 2 Heparin-binding growth factor activity in urine from adult males

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of samples</th>
<th>Age range</th>
<th>Growth factor activity (ng bFGF/ h urine)*</th>
<th>Range (ng bFGF/ h urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder or kidney cancer</td>
<td>18</td>
<td>46-72</td>
<td>65 ± 20‡</td>
<td>0-250</td>
</tr>
<tr>
<td>History of bladder or kidney cancer</td>
<td>6</td>
<td>58-69</td>
<td>3 ± 3‡</td>
<td>0-15</td>
</tr>
<tr>
<td>Benign disease</td>
<td>12</td>
<td>23-84</td>
<td>8 ± 3‡</td>
<td>0-32</td>
</tr>
<tr>
<td>Healthy male controls</td>
<td>14</td>
<td>23-39</td>
<td>3 ± 3‡</td>
<td>0-10</td>
</tr>
</tbody>
</table>

* Heparin-binding growth factor activity was determined in a 5-h urine sample by measuring the ability of 3 M NaCl eluates from heparin-Sepharose columns to stimulate incorporation of [3H]thymidine into confluent BALB/c 3T3 cells.

The results from each sample were averaged with the entire group. Three samples were tested over several months from another patient who had a history of bladder cancer, during which time he remained free of disease. The result from each sample was averaged with the entire group of samples. The mean level of growth factor activity in urine from patients with bladder or kidney cancer was equivalent to 65 ± 20 ng of bFGF-like activity/h of urine production (Table 2). Urine from all 7 patients with kidney cancer and 6 of 11 (55%) of the urine samples from patients with bladder cancer contained activity equivalent to more than 20 ng of bFGF-like activity/h of urine production (Fig. 4). The lowest levels of growth factor activity were found in urine from patients with the smallest tumors.

FGF-like activity was measured in urine samples from three other groups of patients which included healthy male controls, patients with a benign disease, or a history of bladder or kidney cancer (Table 2). Six voided urine samples were tested from four patients who had a history of bladder cancer. None of these patients had evidence of a tumor at the time of urine collection. The mean age of these patients was 64. The mean level of growth factor activity in urine samples from these latter four patients was equivalent to 3 ± 3 ng of bFGF-like activity/h of urine which was significantly lower than the mean level of heparin-binding growth factor activity in urine from bladder and kidney cancer patients (P < 0.005). All of these samples contained less than the equivalent of 20 ng of bFGF-like activity/h of urine production (Fig. 4).

Fourteen samples from 5 healthy individuals, whose mean age was 29, contained significantly less heparin-binding growth factor activity [3 ± 0 (SD) ng of bFGF-like activity/h of urine production] than urine from patients in the bladder/kidney cancer group (Table 2). The coefficient of variation for replicate samples from these five healthy patients ranged from 3 to 8%. All 14 urine samples contained less than the equivalent of 20 ng of bFGF-like activity/h of urine production (Fig. 4).

We also tested 12 urine specimens from 11 patients with benign disease. The diagnoses of these patients included Crohn's disease (n = 3), benign prostatic hypertrophy, nephrolithiasis, testicular pain, benign renal cyst, inguinal hernia, benign renal tumor, cecal adenoma, peritonitis, and chronic renal failure. The mean and median ages of these patients were 57 and 66, respectively. The mean level of heparin-binding growth factor activity in urine from these individuals was equivalent to 8 ± 3 ng of bFGF-like activity/h of urine production (Table 1). Only 2 of 12 (17%) of the samples contained more
other known growth factors. For example, acidic FGF has a required, however, to establish the identity of this protein. Amino acid analysis data will be related to basic FGF. These results show that the tumor-derived growth factor possesses live of the properties that are characteristic of the angiogenic factor, basic FGF. These include (a) a strong affinity for heparin-Sepharose, (b) the ability to stimulate BCE cell proliferation, (c) stimulation of DNA synthesis and (d) heparin binding growth factor activity in individual urine samples.

DISCUSSION

In previous studies, we demonstrated that human and mouse transitional cell carcinoma possesses angiogenic activity (24, 25). However, the substances responsible for this effect were not completely characterized. In this study, we have shown that a protein present in extracts of human bladder tumor cells and kidney tumors possesses five of the properties that are characteristic of the angiogenic factor, basic FGF. These include (a) similar affinity for heparin-Sepharose, (b) the ability to stimulate BCE cell proliferation, (c) stimulation of DNA synthesis in BALB/c 3T3 cells, (d) molecular weight of approximately 17,000, and (e) recognition by anti-basic FGF (peptide) antibodies. These results show that the tumor-derived growth factors are related to basic FGF. Amino acid analysis data will be required, however, to establish the identity of this protein.

These data indicate that the heparin-binding growth factors found in bladder and kidney tumors are probably unrelated to other known growth factors. For example, acidic FGF has a 50% sequence similarity with basic FGF (8, 11, 26). However, unlike the tumor-derived growth factors, acidic FGF does not cross-react with anti-bFGF antiserum. TGF-α and EGF are two other angiogenic factors (27) that have been found in urine (28, 29). However, both of these factors have lower molecular weights (approximately 6,000) (30, 31) and lower affinity for heparin-Sepharose (15) than the tumor-derived factors. The bladder and kidney tumor-derived growth factors also differ from the cationic growth factor, PDGF, in affinity for heparin-Sepharose and ability to stimulate BCE cells; PDGF is eluted from heparin-Sepharose by 0.6 M salt (15), and it does not stimulate BCE cell proliferation. Furthermore, the subunits of PDGF, which are approximately the same molecular weight as the tumor growth factor (32), lack growth factor activity for 3T3 cells. Thus, bladder and kidney tumor-derived growth factors share many of the features of bFGF, and they do not resemble any other known growth factors.

Urine from patients with bladder or kidney cancer also contained growth factor activity that is similar to FGF. Like the bladder tumor-derived growth factor, urinary growth factor activity had a strong affinity for heparin, and it exhibited mitogenic activity in the BCE and BALB/c 3T3 cell growth factor assays. However, it is premature to conclude that the urinary growth factor is identical to bFGF. In contrast to the growth factors found in bladder and kidney tumors, the urinary-derived growth factor was not recognized by bFGF antiserum on Western blot analysis. The absence of a reaction may have been due to insufficient protein because of losses during concentration. It is also conceivable that the urinary factor is related, but not identical, to basic FGF. Recently, a gene that encodes a protein with only partial homology to bFGF was isolated from human bladder cancer cells (33), suggesting that bladder tumors produce more than one class of FGF-related molecules, only one of which may be released into urine.

The large difference in heparin-binding growth factor activity in pooled urine from controls and patients with bladder cancer led us to measure the level of growth factor in individual urine samples. We found that the differences in growth factor activity detected in pooled samples were also observable in the individual samples. Seventy-two percent of the urine samples from patients with bladder or kidney cancer contained elevated levels (more than the equivalent of 20 ng of bFGF-like activity/h of urine collection) of growth factor activity, whereas only 7% of the samples from controls contained this level of growth factor activity.

Urinary FGF-like Activity (ng/hr)

Fig. 4. Heparin-binding growth factor activity in individual urine samples. Five-h urine collections were absorbed onto heparin-Sepharose, and the gel was poured into columns. The columns were washed with 0.6 M NaCl, and growth factor was eluted with 3.0 M salt. The samples were tested in duplicate for the ability to stimulate [3H]thymidine incorporation in BALB/c 3T3 cells. The results are expressed as ng of basic FGF produced per h.

Downloaded from cancerres.aacrjournals.org on April 15, 2017. © 1988 American Association for Cancer Research.
like activity were detected in urine from some patients with catheters in the bladder. In addition, the menstrual cycle may influence urinary excretion of bFGF, as suggested by marked variation in levels among young healthy women. Furthermore, urine from some patients with cancers outside the urinary tract may also contain elevated levels of this factor. The impact of these conditions on urinary bFGF levels is being studied in more detail.

In summary, we have shown that carcinoma of the bladder and kidney contains a heparin-binding growth factor which is similar to basic FGF. This factor may partly account for the angiogenic activity of these tumors. Moreover, this study shows that urine from patients with bladder or kidney cancer contains more FGF-like activity than urine from healthy individuals.

ACKNOWLEDGMENTS

We thank Dr. Michel Boileau and Dr. Richard Williams for providing kidney tumor tissue; Dr. Michael Klagsbrun for helpful discussions; Maria Rivera, Tom Weyrich, and Paul Keller for technical assistance; and Diane Torrey for clerical assistance.

REFERENCES


Unpublished results.
Increased Levels of Fibroblast Growth Factor-like Activity in Urine from Patients with Bladder or Kidney Cancer

Gerald W. Chodak, Verne Hospelhorn, Sheila M. Judge, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/8/2083

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.