Diagnostic and Prognostic Role of Basic Fibroblast Growth Factor in Wilms’ Tumor Patients

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ABSTRACT

Basic fibroblast growth factor (bFGF) is a potent angiogenic peptide implicated in the growth and metastasis of solid tumors. Elevated concentrations of bFGF have been found in the urine of patients with bladder, prostate, and renal tumors. Furthermore, urinary bFGF levels have been shown to correlate with extent of disease. In order to test the utility of urinary bFGF as a Wilms’ tumor marker, we measured bFGF levels in preoperative and postoperative urine samples from 97 patients with Wilms’ tumor. Preoperative urine samples (n = 97), early postoperative samples obtained from 1 to 3 weeks after surgery (n = 43), and late postoperative samples obtained from 1 to 6 months after surgery (n = 66) were collected from Wilms’ tumor patients at 30 institutions between 1989 and 1993. Urine samples from age-matched controls (n = 17) were also obtained. The bFGF levels were determined in duplicate by a competitive sandwich ELISA capable of measuring bFGF at the pg/ml level. Samples were normalized for creatinine content. Urinary bFGF was elevated in 42% of preoperative samples when compared to controls (>90th percentile of normal). Patients with stage III, IV, and V disease had significantly higher preoperative levels of urinary bFGF when compared to patients with stage I and II disease (P < 0.01). Patients with relapse or persistent disease had significantly elevated late postoperative bFGF levels when compared to disease-free patients and controls (P < 0.05). Thus, in patients with Wilms’ tumor, elevated preoperative urinary bFGF levels raise the suspicion of aggressive disease while elevated postoperative levels may indicate recurrence or persistence of disease. These data suggest that bFGF is a biological marker for Wilms’ tumor and may have a role in the evaluation of patients with this disease.

INTRODUCTION

Angiogenesis is crucial to the growth and metastasis of solid tumors (1). One angiogenic peptide, bFGF, is widely distributed among normal and neoplastic tissues (2). bFGF is a 146-amino acid single-chain protein that stimulates the growth of capillary endothelial cells and has been shown to be elevated in the serum and urine of cancer patients (3, 4). Urinary bFGF levels correlated significantly with extent and status of bladder cancer (5) and were also elevated (above the 90th percentile of normal levels) in 10–64% of adult patients with neoplasms, including breast, lung, prostate, colon, and kidney cancer. In these patients, the extent of disease also correlated significantly with urinary bFGF levels (6).

Wilms’ tumor is the most common pediatric renal cancer in North America (7). Tumor markers for Wilms’ have been sought to enhance early detection of the tumor and to improve patient postoperative surveillance. Erythropoietin, neuron-specific enolase, renin, hyaluronic acid, hyaluronic acid-stimulating activity, and hyaluronidase have all been studied as Wilms’ tumor markers (8, 9). In order to test the utility of urinary bFGF as a Wilms’ tumor marker, we measured urinary bFGF with a sandwich ELISA in 206 preoperative and postoperative urine samples from 97 patients with Wilms’ tumor.

PATIENTS AND METHODS

Clinical Material. Preoperative urine samples (n = 97), early postoperative samples obtained from 1 to 3 weeks after surgery (n = 43), and late postoperative samples obtained from 1 to 6 months after surgery (n = 66) were collected from patients at 30 institutions between 1989 and 1993. The majority of preoperative urine samples were obtained as a clean void before the surgery as a routine part of the patient’s work-up. On rare occasions, urine was collected in a sterile setting from an indwelling catheter placed right before surgery. Voided urine samples were obtained in the early postoperative and late postoperative period as part of the patient’s routine outpatient follow-up. Preoperative serum samples (n = 48) and postoperative serum samples obtained from 1 to 6 weeks after surgery (n = 35) were also obtained. Voided urine samples (n = 17) were collected from healthy age-matched controls. All samples were stored at −80°C until assayed. Each patient’s tumor stage, histology, adjuvant therapy, and clinical course was obtained from the National Wilms’ Tumor Study Registry. Tumors were staged according to the Fourth National Wilms’ Tumor Study (Table 1). Relapse was defined by evidence of newly diagnosed tumor on follow-up physical examination or radiological studies. Persistent disease was defined as patients with stage IV or

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3 The abbreviation used is: bFGF, basic fibroblast growth factor.
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Unresectable disease Stage 3
bFGF/g creatinine. Urinary creatinine levels were determined between assays were 6.4% and 4.8%, respectively. All urine samples were run in duplicate. The coefficients of variation within and bFGF levels was measured at 492 nm in a Titertek Multiskan unbound antibody-enzyme reagent, a substrate and amplifier during the first incubation. Following a wash to remove any unbound protein, an enzyme-linked polyclonal antibody specific for bFGF was added to the wells to "sandwich" the bFGF immobilized during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate and amplifier solution were added to the wells. Color change proportional to any bFGF in the sample was bound by the immobilized antibodies. After washing away any unbound proteins, an enzyme-linked polyclonal antibody specific for bFGF was added to the wells to 'sandwich' the bFGF immobilized during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate and amplifier solution were added to the wells. Color change proportional to bFGF levels was measured at 492 nm in a Titertek Multiskan Plus spectrophotometer (Lab Systems, Helsinki). All samples were run in duplicate. The coefficients of variation within and between assays were 6.4% and 4.8%, respectively. All urine samples were normalized by creatinine and expressed in pg bFGF/g creatinine. Urinary creatinine levels were determined based on the Jaffe reaction (Sigma Diagnostics, St. Louis, MO).

Statistical Analysis. Since bFGF was not normally distributed, we present median and 90th percentile levels. Ranges were presented from the 10th percentile to the 90th percentile. Differences between groups were analyzed by the Wilcoxon rank-sum test for unpaired observations. Kaplan-Meier survival curves were fit for the patients who had follow-up, and curves were compared with the log rank test. A P < 0.05 was considered statistically significant.

RESULTS
The control population had a median bFGF level of 207 pg/g with a 90th percentile of 1150 pg/g. Elevated bFGF levels were defined to be greater than the 90th percentile value in the normal control subjects. On the basis of this cutoff, 42% of preoperative urine samples had elevated bFGF levels. The median level of the preoperative urine samples was 540 pg/g with the 90th percentile of 5318 pg/g. The early postoperative urine samples had a median of 321 pg/g with a 90th percentile of 4960 pg/g while the late postoperative urine samples had a median of 12 pg/g and a 90th percentile of 1825 pg/g (Fig. 1). As a group, the preoperative samples were not statistically different from controls. There was no statistical difference found between the postoperative groups (early and late) and controls.

V disease. Disease-free patients were defined as having no evidence of disease on routine follow-up.

bFGF Determination. A sandwich ELISA was used to measure bFGF at the pg/ml level (R & D Systems, Minneapolis, MN). Murine mAbs specific for bFGF were coated on a 96-well polystyrene microtiter plate. Samples and standard concentration solutions were added to the plate and incubated overnight, during which time any bFGF in the sample was bound by the immobilized antibodies. After washing away any unbound proteins, an enzyme-linked polyclonal antibody specific for bFGF was added to the wells to ‘sandwich’ the bFGF immobilized during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate and amplifier solution were added to the wells. Color change proportional to bFGF levels was measured at 492 nm in a Titertek Multiskan Plus spectrophotometer (Lab Systems, Helsinki). All samples were run in duplicate. The coefficients of variation within and between assays were 6.4% and 4.8%, respectively. All urine samples were normalized by creatinine and expressed in pg bFGF/g creatinine. Urinary creatinine levels were determined based on the Jaffe reaction (Sigma Diagnostics, St. Louis, MO).

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Preoperative urine levels did correlate with staging (Fig. 2). Only 22% of patients with stage I or II disease had elevated urinary bFGF levels whereas 64% of patients with stage III, IV, or V disease had elevated urinary bFGF levels. Patients with stage I or II disease had an elevated urinary bFGF level of 73 pg/g with a 90th percentile of 2916 pg/g, which was not significantly different from controls. Patients with stage III, IV, or V disease had a median urinary bFGF level of 1852 pg/g with a 90th percentile of 9122 pg/g. This was significantly higher than the level of control patients (P < 0.01). No statistical difference in urinary bFGF levels was found between patients with favorable and anaplastic Wilms' tumor histology.

In the late postoperative period, patients with relapse or persistent disease had a median urinary bFGF level of 1095 pg/g and a 90th percentile of 4604 pg/g, which was significantly higher than the levels found in disease-free patients (P < 0.05) and controls (P < 0.05). Disease-free patients had a median urinary bFGF level of 11 pg/g and a 90th percentile of 780 pg/g (Fig. 3). Kaplan-Meier survival curves demonstrated that patients with normal preoperative urinary bFGF had a disease-free rate of 76.7% whereas patients with elevated preoperative uri-
Urinary bFGF had a disease-free rate of 66.6% after 2 years (data not shown). This difference was not found to be statistically significant.

Preoperative serum samples had a median of 7.56 pg bFGF/ml with a range of 1.67–28.86 pg/ml. Postoperative serum levels had a median of 6.85 pg bFGF/ml with a range of 1.56–16.9 pg/ml. These are in contrast to the normal median adult level reported by R & D Systems of 1.75 pg bFGF/ml and the associated range of 0.6–6.4 pg/ml (10). No significant difference was found between preoperative and postoperative serum levels. No correlation was found between a patient’s serum bFGF level and the corresponding urinary bFGF level.

**DISCUSSION**

Urinary bFGF is a marker for Wilms’ tumor. Preoperative urine samples from patients with Wilms’ tumor demonstrated elevated bFGF levels in 42% of cases. Elevated levels of urinary bFGF levels correlated significantly with tumor stage, and patients with relapse or persistence demonstrated significantly higher late postoperative urinary bFGF levels when compared to disease-free patients.

There is considerable experimental evidence to indicate that tumor growth is dependent on angiogenesis (11). Although the initiation of angiogenic activity by a given tumor is not well understood, it is clear that the switch to an angiogenic phenotype demarcates two stages in the development of a tumor: the prevascular phase and the vascular phase (12). The prevascular phase, which has been examined in carcinoma of the cervix, bladder, and breast (13–15), may persist for years and is associated with limited tumor growth. In contrast, the vascular phase is followed by rapid tumor growth, bleeding, and metastasis. This theory has also been demonstrated experimentally. For instance, the growth of tumors implanted in the avascular cornea is slow and linear before vascularization but nearly exponential after vascularization (16).

The first quantitative evidence that the intensity of angiogenesis in a human tumor could predict the probability of metastasis was observed in studies of patients with cutaneous melanoma (17, 18). In these patients, there was a clear distinction between a stage without neovascularization, which correlated with a paucity of metastasis, and a stage with increasing neovascularization, which correlated with a rising rate of metastasis. An association between tumor angiogenesis and metastasis has also been observed in invasive breast carcinoma (12). Folkman (19) has suggested that an association between ‘angiogenic peptides’ such as bFGF and tumor metastasis may also exist. Our study demonstrates that there is a correlation between urinary bFGF levels and tumor staging in Wilms’ tumor. Patients with unresectable disease (stage III, IV, or V) had significantly higher bFGF levels than patients with resectable disease (stage I or II).

That angiogenic peptides could serve as tumor markers was first demonstrated when the urine of patients with bladder and renal cancers was shown to stimulate migration of capillary endothelial cells (20). The capillary endothelial cell growth factor found in the urine of patients with bladder and renal
cancers was subsequently identified to be bFGF (21). Recently, urinary bFGF levels have been shown to be elevated in adult patients with a variety of cancers, including breast, lung, and kidney (5, 6). Our study indicates that urinary bFGF is elevated preoperatively in over 40% of patients with Wilms’ tumor.

Elevated urinary bFGF in patients with Wilms’ tumor could originate from either the neovascular endothelium or the tumor cells. For a tumor to metastasize, tumor cells must gain access to the vasculature by local angiogenesis (12). Vascular endothelial cells synthesize bFGF (5), and newly proliferating capillary endothelial cells that become part of the tumor could contribute to the high urinary bFGF levels seen in patients with Wilms’ tumor. Another possible source of bFGF may be from the tumor itself. Using a tumor-bearing mouse model, bFGF from endothelial and tumor cells was distinguished, and urinary bFGF appeared to originate exclusively from the tumor cells (22).

The metabolism of bFGF may explain why there was no correlation between serum and urinary bFGF levels. In mice, bFGF has a short serum half-life of 30 seconds and is sequestered rapidly in the kidney (23, 24). If the tumor is releasing bFGF intermittently into the serum, elevated levels of serum bFGF would be difficult to detect. Furthermore, since bFGF is sequestered in the kidney, it is reasonable that the kidney may preferentially secrete bFGF into the urine which would account for the markedly elevated levels found in some of our patients with Wilms’ tumor.

The low sensitivity and specificity of urinary bFGF would not allow this test to be an effective childhood screening tool for Wilms’ tumor. However, in a select high-risk population of patients, such as those with a positive family history of Wilms’ tumor, aniridia, Beckwith-Wiedemann syndrome, nephroblastomatosis, or hemihypertrophy, bFGF levels may be a useful early indicator of Wilms’ tumor. Preoperative urinary bFGF levels may also assist in determining the aggressiveness of the tumor.

Although treated Wilms’ tumor has a greater than 80% survival rate in North America (25), there remains a risk of metachronous involvement of the opposite kidney or unrecognized persistence of disease. Despite close follow-up, small lesions which escape clinical and radiological detection can develop. Currently, there is no effective tumor marker for Wilms’ tumor. Urinary bFGF levels may be a simple and noninvasive means for early detection of relapse and predictive of recurring disease. In our study, patients with an elevated late postoperative urinary bFGF level had a higher probability of having relapse or persistent disease. Six of the eight patients with elevated late postoperative urinary bFGF had either persistent disease or developed relapse within 2 years. A tumor marker such as bFGF could decrease the number of missed recurrences; elevated postoperative bFGF levels would suggest an increased risk of relapse or persistence, indicating a need for reassessment and possible adjuvant treatment. Thus, analysis of urinary bFGF may provide an additional quick and convenient tool for the detection of Wilms’ tumor relapse or persistence.

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