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Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor Urine Levels as Predictors of Outcome in Hormone-refractory Prostate Cancer Patients: A Cancer and Leukemia Group B Study[1]

Robert A. Bok,[2] Susan Halabi, David T. Fei, Carlos R. Rodriquez, Daniel F. Hayes, Nicholas J. Vogelzang, Philip Kantoff, Marc A. Shuman, and Eric J. Small

University of California at San Francisco, San Francisco, California 94143-0324 [R. A. B., M. A. S., E. J. S.]; CALGB Statistical Center, Durham, NC 27710 [S. H.]; Genentech, Inc., South San Francisco, California 94080 [D. T. F., C. R. R.]; Georgetown University Medical Center, Lombardi Cancer Center, Washington, DC 20007 [D. F. H.]; University of Chicago Medical Center, Chicago, Illinois 60637 [N. J. V.]; and Dana Farber Cancer Institute, Boston, Massachusetts 02115 [P. K.]

ABSTRACT

Better prognostic markers are needed for hormone-refractory prostate cancer (HRPC) patients. No single biochemical or clinical parameter can reliably predict patient response to therapy or rapidity of disease progression. Peptide factors involved in major cancer growth pathways, such as tumor angiogenesis, are attractive candidates as markers of low- and high-risk HRPC patients. We analyzed prospectively collected urine specimens from 100 of 390 HRPC patients undergoing therapy with the growth factor antagonist suramin as part of CALGB 9480. Levels of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) were assessed from day 1 of therapy (D1) and day 29 (D29) urine samples from this subset of 100 randomly selected patients. Growth factor levels were determined by standardized ELISA microtiter plate assays from a commercial (bFGF) or proprietary (VEGF) source. Pretreatment urine VEGF levels were predictive of survival. In univariate analysis, patients whose baseline urine VEGF level was ≤28 pg/ml (the median level) had an average survival of 17 months; those with baseline VEGF >28 pg/ml had a significantly shorter survival of 10 months ($P = 0.024$). This difference corresponded to a 60% increased risk of dying for the higher urine VEGF patients (hazard ratio, 1.62; $P = 0.03$) and remained significant in multivariate analysis (hazard ratio, 1.72, $P = 0.02$). No significant correlations between urine bFGF level or change in bFGF levels and survival were found. These results support the notion that certain peptide growth factor-mediated, mitogenic pathways are important in HRPC and that their levels can predict outcome.

INTRODUCTION

HRPC[3] is a heterogeneous disease in which few prognostic markers exist. A posttreatment decline in PSA has been identified as an intermediate marker of outcome (1, 2). Pretreatment variables that appear to predict survival include performance status, Hgb, and weight loss (3). A prognostic formula based on PSA changes, Hgb level, and weight loss has also been derived (4). Nevertheless, better prognostic markers are required to identify both high- and low-risk HRPC patients for whom therapy could be more specifically tailored.

The measurement of angiogenic growth factor levels in urine as prognostic factors in cancer was first studied by Folkman and colleagues in the early 1990s. Ngyuyen *et al.* (5) found elevated levels of bFGF in urine of bladder cancer patients and later extended the observation to lung, breast, lymphoma, and prostate cancer patients (6). More recently, VEGF urine levels have been evaluated in bladder cancer patients and were found to correlate with the tumor recurrence rate (7).

CALGB 9480 was a multicenter, Phase III trial evaluating suramin administered at three different dose levels in HRPC patients. On this trial, which closed to accrual in July 1998, all patients underwent prospective comprehensive sampling of urine and plasma for VEGF and bFGF levels. The goals of this prospectively designed study were to assess clinical response and toxicity for three different suramin doses, to measure population pharmacokinetics, and to evaluate urine levels of selected growth factors known to interact with suramin. In particular, it was hypothesized that high pretreatment VEGF and bFGF urine levels would be markers of worse outcome. Because of the complexity of interactions between suramin, growth factors, and their receptors, it was unknown how the growth factor levels would change after treatment with suramin. Most importantly, this large Phase III study allowed the prospective collection of urine samples from a reasonably homogeneous group of HRPC patients treated on the same clinical trial.

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[2] To whom requests for reprints should be addressed, at University of California at San Francisco, Comprehensive Cancer Center, 1600 Divisadero, Third Floor, San Francisco, CA 94143-0324. Phone: (415) 885-7838; Fax: (415) 474-9173; E-mail: bokr@medicine.ucsf.edu.

[3] The abbreviations used are: HRPC, hormone-refractory prostate cancer; PSA, prostate-specific antigen; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; CALGB, Cancer and Leukemia Group B; D1, day 1; D29, day 29; Hgb, hemoglobin; HR, hazard ratio; CI, confidence interval.

MATERIALS AND METHODS

Patient Specimens. Urine samples from the 390 patients enrolled on CALGB 9480 were collected on days 1, 2, 8, 9, 29, and 65. Specimens were frozen after collection, shipped on dry ice by overnight mail to a central repository, and stored at −70°C until the time of assay. One hundred patients were randomly selected from the cohort of 390, and their D1 and D29 urine specimens were thawed for growth factor analysis. The present analysis was undertaken with samples from only a portion of the entire patient cohort as an exploratory, hypothesis-generating study. After thawing, specimens were centrifuged at 850 × *g* for 20 min to remove sediment, as recommended for bFGF assessment (5). Aliquots were removed for duplicate to quadruplicate ELISA assays. bFGF levels were determined using a commercially available, high-sensitivity assay plate (Quantikine HS) from R&D Systems (Minneapolis, MN). VEGF levels were determined using a fluorometric ELISA assay developed at Genentech, Inc., as described by Rodriguez *et al.* (8). Aliquots of each urine sample were also used for creatinine level determination, using an enzymatic alkaline picrate assay (9). Standardization for creatinine content was obtained by dividing the bFGF or VEGF concentration of a particular sample by its creatinine concentration, reported as pg/g.

Statistical Analysis. Pretreatment urine bFGF and VEGF levels were used to dichotomize patients into low- and high-level groups based on the median values of the urinary bFGF and VEGF measurements. The Kaplan-Meier product-limit method (10) was used to estimate the survival duration in the low and high growth factor level groups of patients. The log-rank statistical method was used to test for differences in the distribution of the survival times between the two baseline VEGF and bFGF groups. Baseline urine bFGF and VEGF levels and changes in these growth factor levels from D1 to D29 were analyzed with regard to survival. Univariate analyses were carried out for baseline (D1) VEGF and bFGF as well as various changes in urine levels relative to D29, including difference between D29 and D1 levels, absolute reduction in D29 level, 15 and 20% reduction in levels at D29, and ratio of D29:D1 levels. Analyses were performed using growth factor levels as continuous variables and as categorical variables with certain cut-off points (*e.g.*, 75, 50, 20, and 15% reduction in growth factor level). Absolute growth factor levels in urine as well as growth factor levels corrected for creatinine content were analyzed. In addition, the proportional hazards regression model (11) was used to assess the prognostic importance of baseline VEGF and bFGF levels, adjust for potentially confounding pretreatment variables reported in other series to be of prognostic significance, including baseline PSA, alkaline phosphatase, Hgb serum creatinine, performance status, and weight loss. All tests were performed using a two-sided α level of 0.05.

RESULTS

Patient Characteristics. The median age of patients assayed was 70. Eighty-five % had an Eastern Cooperative Oncology Group performance status of 0 or 1; 15% had an Eastern Cooperative Oncology Group performance status of 2. All had metastatic disease: 90% had bone metastases, and 39% had bidimensionally measurable disease. The median pretreatment PSA and alkaline phosphatase levels were 131 ng/ml and 172 IU, respectively. The baseline characteristics of the 100 patients in this study were very similar to those of the CALGB 9480 patients as a whole (Table 1). The median age of patients, the distribution of race, the distribution of metastases, performance status distribution, medians of various blood chemistries (Hgb, PSA, alkaline phosphatase, and serum creatinine), and, finally, the distribution of patients between the three treatment arms were essentially the same in the assayed subset and the entire group of CALGB 9480 patients.

Table 1 *Baseline characteristics of 100 patients assayed for urine bFGF and VEGF levels and entire CALGB 9480 population*

	Assayed patient subset (n = 100)	Entire 9480 group (n = 390)
Median age, yr	70 (63–76)	70 (64–75)
Race, % white	84%	81%
Metastases		
% with bone	90	91
% with lymph node	29	32
% with lung	6	6
% with liver	6	6
% with measurable disease	39	36
Performance status		
0–1	85%	88%
2	15%	12%
Laboratory values		
Median Hgb (g/dl)[a]	13 (12–14)	13 (11–14)
Median PSA (ng/ml)[a]	131 (42–321)	128 (49–338)
Median alkaline phosphatase (IU/l)[a]	172 (106–271)	164 (99–313)
Median creatinine (mg/dl)[a]	1.0 (1–1)	1.0 (1–1)
Suramin dose		
Low	36%	34%
Medium	32%	33%
High	32%	33%

[a] Interquartile range.

Table 2 *VEGF and bFGF urine levels, D1 and D29: Uncorrected and corrected for creatinine (pg/g)*

	D1 VEGF (pg/ml)	D29 VEGF (pg/ml)	D1 VEGF:CREAT[a]	D29 VEGF:CREAT
Median	28.0	37.9	28900	43700
Mean	45.6	45.6	31500	47400
SD	57.8	33.2	23700	22200
Range	0–488	4.29–210	0–113000	0–150000
	D1 bFGF (pg/ml)	D29 bFGF (pg/ml)	D1 bFGF:CREAT	D29 bFGF:CREAT
Median	0.506	1.39	526	1620
Mean	1.68	2.58	1440	3190
SD	4.30	3.60	2600	5040
Range	0.085–31.1	0.183–27.4	45–17700	121–43200

[a] CREAT, creatinine.

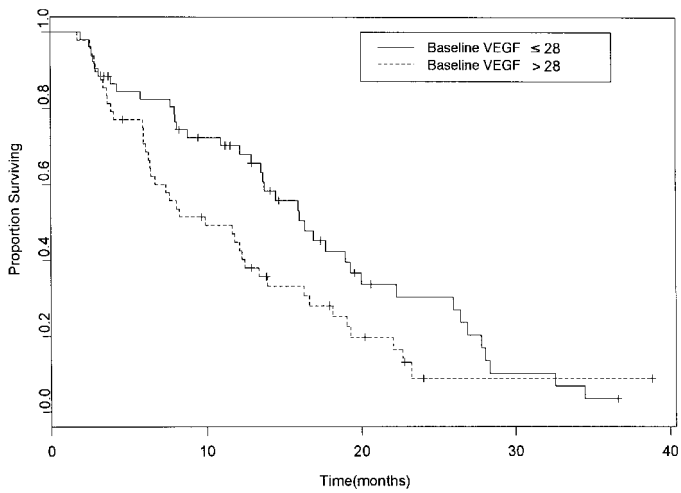

Fig. 1. Kaplan-Meier survival analysis for baseline VEGF using median VEGF as a cutpoint. Lower baseline urinary VEGF levels are associated with improved survival (P = 0.024).

Growth Factor Levels. The median VEGF urine levels on D1 and D29 were 28.0 and 37.9 pg/ml, respectively. The mean VEGF levels on D1 and D29 were 45.6 and 45.2 pg/ml, respectively. The median bFGF levels on D1 and D29 were 0.506 and 1.39 pg/ml, respectively, whereas the corresponding mean bFGF levels were 1.68 and 2.58 pg/ml. Thus, for the two sample dates tested in this suramin-treated patient population, the urinary growth factor levels in the group as a whole increased from D1 to D29 (Table 2). Urine creatinine levels were also determined on all samples, as had been described previously (5). However, correction for creatinine did not improve any of the associations examined.

Association of Growth Factor Levels with Survival: Univariate Analysis. In univariate analysis for VEGF, only two parameters showed statistically significant associations with survival: (*a*) baseline urine VEGF using the median level as cutpoint (Fig. 1);

Table 3 *Univariate analysis*

Survival analysis	HR (95% CI)	*P*
A. Urine VEGF		
Baseline VEGF (>28 *vs.* ≤28 pg/ml)	1.66 (1.06–2.59)	0.03
15% reduction in VEGF D29 from baseline	1.62 (1.01–2.62)	0.05
Baseline VEGF (D1) (continuous)	1.00 (1.00–1.01)	0.07
Ratio D29:D1 VEGF (continuous)	0.98 (0.95–1.01)	0.11
Reduction in VEGF D29 from D1 (continuous)	1.03 (0.99–1.06)	0.11
Difference between VEGF D29 and D1	1.00 (0.99–1.06)	0.15
Ratio D29:D1 VEGF (>1 *vs.* ≤1)	0.75 (0.47–1.18)	0.21
B. Urine bFGF		
Ratio D29:D1 bFGF (>1 *vs.* ≤1)	0.63 (0.38–1.07)	0.08
bFGF (>0.5 *vs.* ≤0.5)	1.39 (0.89–2.17)	0.15
Baseline bFGF (D1) (continuous)	1.02 (0.97–1.07)	0.48
15% reduction in bFGF, D29 from D1	1.22 (0.68–2.19)	0.50
Reduction in bFGF, D29 from D1 (continuous)	1.00 (0.97–1.04)	0.83
Ratio D29:D1 bFGF (continuous)	1.00 (0.96–1.03)	0.83
Difference between bFGF, D29 and D1	1.00 (0.95–1.05)	0.97

and (*b*) decrease in urine VEGF at D29 using a 15% reduction cutpoint (Table 3A). A baseline urine VEGF level ≤28 pg/ml was associated with improved survival, with a HR of 1.66, $P = 0.03$ (Fig. 1). With log-rank testing, this difference corresponded to a median survival of 17 months for patients with baseline urine VEGF ≤28 pg/ml compared with a median survival of 10 months for patients with baseline urine VEGF >28 pg/ml (Table 4A). Univariate analysis for bFGF showed no statistically significant association with survival in any of eight categories examined (Table 3B): baseline bFGF (continuous); bFGF using the median level as cutpoint; difference between D29 and D21 bFGF; reduction in bFGF D29 from baseline (continuous); 15% reduction in bFGF D29 from baseline; 20% reduction in bFGF D29 from baseline; ratio D29:D1 bFGF (continuous); and ratio D29:D1 bFGF (>1 *versus* ≤1). Only the ratio D29:D1 using 1 as cutpoint approached statistical significance (HR, 0.63; $P = 0.08$).

Multivariate Analysis. Multivariate analyses were performed with VEGF and bFGF predicting survival, adjusting for weight loss in the past 6 months, baseline PSA, performance status, plasma Hgb, serum alkaline phosphatase, measurable disease, and race. Pretreatment VEGF was an independent prognostic factor, with high levels of VEGF predicting poor survival (Table 5). The HR was 1.72 for patients with a urine VEGF level >28 pg/ml compared with patients with a level ≤28 pg/ml ($P = 0.020$). The strongest prognostic factor was performance status, with a HR of 2.45 ($P = 0.004$).

DISCUSSION

Few multicenter, prospective investigations have been performed in HRPC patients to evaluate specific prognostic factors. In this study, urine specimens were prospectively collected with the intent of determining bFGF and VEGF levels and assessing their prognostic value in this large Phase III trial. Patients on this trial underwent treatment with suramin, a polysulfonated naphthylurea that disrupts peptide growth factor:receptor associations. Although the exact mechanisms of suramin action are not understood, it clearly antagonizes growth factor:receptor interactions for such ligands as platelet-derived growth factor, bFGF, epidermal growth factor, VEGF and transforming growth factor β (12, 13). Because they are two of the major angiogenic growth factors, bFGF and VEGF were of particular interest in relation to suramin activity.

Table 4 *Median survival as a function of pretreatment VEGF or bFGF urine level*

VEGF	*n*	Median survival (95% CI)	
A. Pretreatment VEGF urine level			
Low (≤28[a])	52	17 mo (14–22)	$P = 0.024$
High (>28)	49	10 mo (6–13)	
B. Pretreatment bFGF urine level			
bFGF	*n*	Median survival (95% CI)	
Low (≤0.5[b])	52	16 mo (9–19)	$P = 0.150$
High (>0.5)	49	13 mo (8–16)	

[a] Median, 28 pg/ml; log-rank test, 5.09 (1 degree of freedom).
[b] Median, 0.5 pg/ml; log-rank test, 2.09 (1 degree of freedom).

In this group of patients with metastatic HRPC treated with suramin, lower baseline urine VEGF levels predicted for increased survival. This is the first demonstration in any cancer, to our knowledge, that urine VEGF levels are predictive of survival. Using the median VEGF level as a cutpoint, patients with >28 pg/ml at baseline had a 70% increase in the HR (risk of dying) compared with those with VEGF ≤28 pg/ml. Median survival for these two groups was 17 and 10 months, respectively ($P = 0.024$). This difference held true in a multivariate analysis as well.

Better prognostic markers are needed to design more rigorous strategies for treating advanced prostate cancer. The prognostic markers that have been identified in HRPC are few, despite investigation of a variety of factors including performance status, weight loss, age, race, site of metastases, Hgb, albumin, lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase, prostatic acid phosphatase, carcinoembryonic antigen, and various measures of PSA (3, 4). Although a number of these variables have shown definite correlations with clinical outcomes, they have not been corroborated in prospective, randomized trials.

Additional efforts have begun to define the utility of specific peptide growth factors as prognostic markers, including insulin growth factor-I (14) and hepatocyte growth factor in the hormone-refractory setting (15). Urine VEGF level has been shown previously to correlate with risk of relapse in bladder cancer patients (7). Several studies have evaluated plasma or serum levels of VEGF in prostate cancer patients (16, 17), as well as other malignancies (18), but failed to demonstrate prognostic significance. More recent reports suggest a correlation between plasma or tissue VEGF levels and survival in other malignancies. Yoshikawa *et al.* (19) examined plasma VEGF levels in 54 gastric carcinoma patients and found a statistically significant association between VEGF level and survival in both univariate and multivariate analyses. They found no survival association with plasma bFGF level. Linderholm *et al.* (20) examined CD31, bFGF, and VEGF

Table 5 *Multivariate survival analysis*

Survival analysis	HR for death (95% CI)	*P*
Performance status (2 *vs.* 0,1)	2.45 (1.34–4.48)	0.004
Baseline VEGF (>28 *vs.* ≤28 pg/ml)	1.72 (1.09–2.71)	0.020
Baseline PSA (>131 *vs.* ≤131 ng/ml)	1.61 (1.02–2.53)	0.040

levels in cytosol extracts of tumor specimens from 827 breast carcinoma patients. In that study, VEGF level but not bFGF was an independent predictor of survival. The findings of the latter two studies are therefore in general agreement with the results of our study, *i.e.*, that higher urine VEGF, but not urine bFGF, levels correlate inversely with survival.

In contrast with the VEGF data, pretreatment bFGF urine levels in this study were not predictive of survival. No statistically significant correlations between bFGF or changes in bFGF urine levels and survival were found. In fact, only an increase in D29 bFGF level *versus* a decrease (D29:D1 >1 *versus* D29:D1 ≤1) approached statistical significance, showing a HR of 0.63 ($P = 0.08$). This trend would suggest that an increase in urine bFGF level might portend a better clinical outcome. Similar associations with bFGF levels have been found in other studies (21).

Interestingly, a <15% reduction in VEGF urine level from D1 to D29 was also significantly associated with increased survival. This correlation was discovered after exploration of various cutpoints for the D1 to D29 changes and may reflect a chance finding. However, it bears some similarity to the trend seen for D29:D1 bFGF ratio noted above. Taken together, these observations suggest that an increase in urine growth factor levels or a very minimal decrease by D29 of suramin treatment may reflect a biological benefit. One could speculate that this is a result of decreased binding of the ligand growth factors with their target receptors, because of suramin therapy, and a concomitant increase in their urinary excretion. The validity of these associations will have to be confirmed by analyses of additional urine samples from patients treated on CALGB 9480.

A potential confounder in the interpretation of peptides/proteins as markers of disease progression or response to therapy is the protective or therapeutic effect that elevation of certain proteins/peptides may have against the disease process. Angiostatin and endostatin are examples in point. These proteolytic fragments of major tissue proteins are increased in cancer patients but have antiangiogenic and thus potential antitumor activity (21). Similarly, PSA levels generally increase with increasing prostate cancer tumor burden, and recent reports suggest that high PSA levels may have antiangiogenic and antitumor activity (22). Consequently, it may be necessary to dissect out the various potentially contradictory contributions of growth factors to cancer cell proliferation, migration, metastases, and angiogenesis. The complexity of these interactions makes it likely that various factors will need to be analyzed in a multivariate analysis in a large group of patients to accurately define their association with clinical outcomes. Our selected assay group, although phenotypically similar to the entire CALGB 9480 group, could represent a unique subset of patients. Full analysis of this group of HRPC patients, therefore, seems warranted and is planned, along with an analysis of posttreatment variables such as PSA change. This present analysis should therefore be viewed as exploratory and hypothesis generating.

In summary, these results are consistent with the notion that certain peptide growth factor-mediated, mitogenic pathways are important in HRPC. The data also suggest that urinary VEGF levels may be able to identify a subset of HRPC patients who have a worse outcome. Therapies targeted at this growth factor pathway are under development (23), and in the future, prospective trials could be designed to enrich for patients who might benefit from such therapies.

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