High Pretreatment Serum Concentration of Basic Fibroblast Growth Factor Is a Predictor of Poor Prognosis in Small Cell Lung Cancer[1]

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Abstract

Basic fibroblast growth factor (bFGF) is a secreted multifunctional cytokine and a potent stimulator of angiogenesis. We measured bFGF concentrations from serum samples taken from 103 patients with small cell lung cancer at the time of diagnosis. Serum concentration of bFGF (S-bFGF) ranged from undetectable to 54 pg/ml (median, 6 pg/ml). S-bFGF was not associated with age, sex, performance status, or stage. A high pretreatment S-bFGF was associated with poor overall survival. The 1- and 2-year survival rates of the patients within the highest quartile of S-bFGF (≥17 pg/ml) were only 26% and 11%, respectively, in contrast to the 49% and 20% 1- and 2-year survival rates of those patients with S-bFGF < 17 pg/ml (*P* = 0.013). The 1- and 2-year survival rates of the patients with extensive-stage disease were 33% and 10%, respectively (*P* = 0.0091). Interestingly, S-bFGF provided additional prognostic information to the stage because the 1- and 2-year survival rates of patients with extensive-stage disease and a high S-bFGF (≥17 pg/ml) were as low as 16% and 5%, respectively (*P* = 0.0026). Similarly, in the multivariate model of survival analysis, patients with both extensive-stage disease and a high S-bFGF (≥17 pg/ml) were found to have a particularly poor prognosis (relative risk of death, 2.1; 95% confidence interval, 1.2–3.6; *P* = 0.0057). We conclude that a high S-bFGF at diagnosis is associated with poor outcome in small cell lung cancer, possibly reflecting active angiogenesis and rapid tumor growth, and may complement prognostic information obtained by staging.

Introduction

Active angiogenesis is a prerequisite for tumor growth beyond a few cubic millimeters in size and also for the dissemination of cancer (1). Angiogenesis is regulated by a balance of various positive and negative angiogenic molecules (2). bFGF,[3] also called fibroblast growth factor-2, is a secreted multifunctional cytokine that acts as a mitogen for endothelial cells and is a potent inducer of angiogenesis *in vivo* (3, 4). The important role of bFGF in tumor angiogenesis has been shown *in vivo* by immunoneutralizing antibodies against bFGF, which inhibit tumor growth in nude mice (5).

High concentrations of bFGF have been detected in the urine or serum of cancer patients (6–12). A high S-bFGF has been found to be associated with a large tumor size in head and neck cancer (13) and with a short tumor volume doubling time in colorectal cancer (14). In CLL, elevated intracellular level of bFGF correlates with stage, and it is also associated with resistance to chemotherapy (15). Recently, we found that a high pretreatment S-bFGF level is a strong predictor of poor prognosis in non-Hodgkin's lymphoma (16, 17). We now wanted to study the possible predictive value of S-bFGF in SCLC, and we measured bFGF concentrations from serum samples taken from 103 patients with SCLC at the time of the diagnosis.

Materials and Methods

Patients. S-bFGF was measured in 103 patients with histologically proven SCLC diagnosed and treated in the Department of Internal Medicine, Helsinki University Central Hospital, between 1990 and 1998. The patients had been participating in a randomized clinical trial to assess the therapeutic value of IFN given concomitantly with chemotherapy and as maintenance therapy (18). The patients were included in the present study if a frozen serum sample taken at the time of the diagnosis and before cancer treatment was available. All patients were treated with combination chemotherapy, consisting of 6 cycles of cisplatin (70 mg/m^2) i.v. on day 1 every 28 days and etoposide (100 mg/m^2) i.v. on days 1, 2, and 3 every 28 days. The patients were randomly assigned to receive chemotherapy alone or 3×10^6 IU i.m. natural leukocyte IFN (Finnferon-Alpha; Finnish Red Cross, Helsinki, Finland) or recombinant IFN-α-2a (Roceron-A, Roche, Basel, Switzerland) concomitantly with chemotherapy from day 1 of cycle 1 until discontinuation of all cancer treatment. In the event of progression at the locoregional site or brain metastases, chemotherapy was stopped, and the patient was treated with radiotherapy, whereas in the event of progression elsewhere, the patient was offered second-line chemotherapy consisting of epirubicin (75 mg/m^2) and ifosfamide (4–5 g/m^2) i.v. on day 1 every 28 days, to a maximum of 6 cycles. Seventy-one (69%) of the patients were men, and the median age was 58 years (range, 41–78 years). Physical examination, a chest X-ray, computed tomography scan, and

Received 1/25/02; revised 1/25/02; accepted 7/5/02.

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[1] Supported by grants from the Sigrid Juselius Foundation, the Finnish Medical Foundation, the Maud Kuistila Memorial Foundation, and the Ella and Georg Ehrnrooth Foundation.

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[3] The abbreviations used are: bFGF, basic fibroblast growth factor; S-bFGF, serum concentration(s) of bFGF; SCLC, small cell lung cancer; RR, relative risk; CI, confidence interval; CLL, chronic lymphocytic leukemia.

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Table 1 Univariate survival analyses of 103 SCLC patients

Cumulative survival from the diagnosis was computed using the product-limit method. The Wilcoxon test was used to compare the different groups.

Factor	12-Month survival	24-Month survival	*P*
WHO performance status			
0	63%	23%	0.0010
1–4	26%	13%	
Stage			
Limited disease	58%	30%	0.0091
Extensive disease	33%	10%	
S-bFGF at diagnosis			
<17 pg/ml	49%	20%	0.013
≥17 pg/ml (the highest quartile)	26%	11%	
Age at diagnosis (yrs)			
≤58	48%	21%	0.23
>58 (Median)	37%	14%	
Sex			
Female	50%	22%	0.57
Male	39%	16%	
Stage & S-bFGF			
Limited-stage disease & <17 pg/ml	59%	31%	0.0026
Extensive-stage disease & ≥17 pg/ml	16%	5%	

routine laboratory tests were performed before randomization. Two (2%) of the patients had stage I disease at diagnosis, 1 (1%) had stage II disease at diagnosis, 37 (36%) had stage III disease at diagnosis, and 63 (61%) had stage IV disease at diagnosis. Stages from I to III were considered "limited disease" (n = 40; 39%), and stage IV (with distant metastasis) was classified as "extensive disease." During the first year of follow-up, 55 (53%) patients died, and by the end of the second year, 85 (83%) patients had succumbed. All surviving patients were followed-up longer than for 24 months.

Serum Samples and bFGF Immunoassay. Peripheral venous blood samples were taken before treatment. The samples were collected in sterile test tubes, centrifuged at 2000 × g for 10 min, and then stored at −20°C. S-bFGF concentrations were determined as S-bFGF immunoreactivity using a quantitative sandwich enzyme immunoassay technique (Quantikine High Sensitivity Human Fibroblast Growth Factor Basic Immunoassay; R&D Systems, Minneapolis, MN) as described previously (16). The system uses a solid phase monoclonal antibody and an enzyme-linked polyclonal antibody raised against recombinant human bFGF. For each analysis, 100 μl of serum were used. All analyses and calibrations were carried out in duplicate. The calibrations on each microtiter plate included recombinant human bFGF standards. Optical densities were determined using a microtiter palate reader (Multiscan RC Type 351; Labsystems, Helsinki, Finland) at 490 nm. The blank was subtracted from the duplicate readings for each standard and sample. A standard curve was created by plotting the logarithm of the mean absorbance of each standard *versus* the logarithm of the cytokine concentration. Concentrations are reported as pg/ml. No association was found between the length of the storage and the bFGF concentration (P > 0.1; Mann-Whitney test). This result is in agreement with our earlier findings (16) and suggests that serum samples can be safely stored at – 20°C for at least 8 years (the study period) without a significant decline in bFGF immunoreactivity.

Statistical Analysis. Statistical analyses were done using the software package StatView 5.01 (SAS Institute Inc., Cary, NC). The Mann-Whitney test was used to compare S-bFGF concentrations in different groups. Cumulative survival was computed according to the product-limit method of Kaplan-Meier from the date of the diagnosis. The Wilcoxon test was used to compare survival of the different subgroups of patients. The relative influence of different variables on survival was studied in multivariate survival analyses using the proportional hazards model of Cox. All Ps are two-tailed.

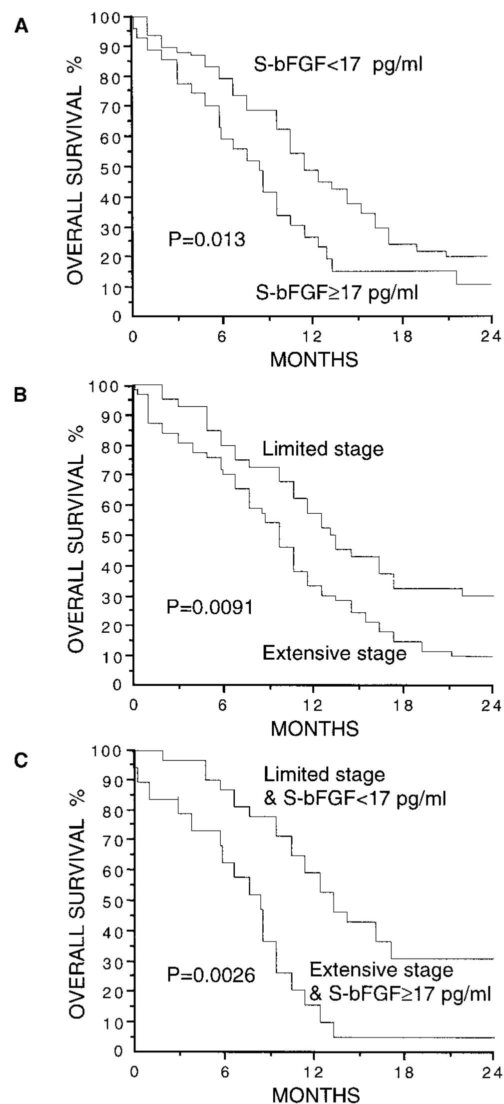

Fig. 1. Overall survival of 103 patients with SCLC by pretreatment S-bFGF (*A*), stage (*B*), and the combination of S-bFGF and stage (*C*). The highest quartile (serum bFGF ≥17 pg/ml) was used as the cutoff value.

Results

S-bFGF in Patients at Diagnosis. S-bFGF concentrations ranged from undetectable to 54 pg/ml (median, 6 pg/ml; mean, 11 pg/ml) among the 103 patients with SCLC. One-fourth of the patients had S-bFGF ≥ 17 pg/ml (the highest quartile). The S-bFGF were not associated with age at diagnosis (tested > median ≤, 58 years), the WHO performance status (0 *versus* 1–4), stage (limited *versus* extensive), or sex (P > 0.05 for all comparisons).

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Table 2 Multivariate survival analyses of 103 SCLC patients

The proportional hazards model of Cox was used.

Factor	RR (e^{β})	95% CI for RR	*P*
Stage			
Extensive disease	1.8	1.1–2.8	0.02
S-bFGF at diagnosis			
≥17 pg/ml (the highest quartile)	1.5	0.9–2.4	0.09
WHO performance status			
1–4	1.4	0.9–2.2	0.12
Extensive stage disease & S-bFGF ≥17 pg/ml	2.1	1.2–3.6	0.0057

S-bFGF and Overall Survival. Several factors correlated strongly with overall survival in univariate survival analyses in the present series (Table 1). Patients with a high S-bFGF at diagnosis had inferior overall survival in comparison with those with lower pretreatment concentration of S-bFGF. The 1- and 2-year survival rates of the patients within the highest quartile of S-bFGF concentrations (S-bFGF ≥ 17 pg/ml) were only 26% and 11%, respectively, in contrast to the 49% and 20% 1- and 2-year survival rates of those patients with S-bFGF < 17 pg/ml ($P = 0.013$; Fig. 1, Table 1). The 2-year survival rate of patients with extensive-stage disease was 10%, in comparison with the 30% 2-year survival rate of the patients with limited-stage disease ($P = 0.0091$; Table 1; Fig. 1). Interestingly, S-bFGF appeared to provide additional prognostic information to the stage because the 2-year survival rate of patients with both a high S-bFGF (≥17 pg/ml) and extensive-stage disease ($n = 19$) was as low as 5%, in contrast to the 31% 2-year survival rate of those 32 patients with both a low S-bFGF and limited-stage disease ($P = 0.0026$; Table 1; Fig. 1).

S-bFGF in Multivariate Survival Analyses. To find out whether high pretreatment S-bFGF has an independent influence on survival, it was entered in multivariate analyses together with performance status and stage. In the proportional hazards model of Cox, extensive stage surfaced as the only factor having independent influence on survival (the RR of death ($e\beta$), 1.8; 95% CI, 1.1–2.8; $P = 0.02$; Table 2). However, similar to the results of the univariate analyses, S-bFGF provided additional prognostic information to the stage in the multivariate model of survivalas well. The highest independent prognostic power in the model was obtained when stage and S-bFGF were combined: again, the patients with extensive-stage disease and high S-bFGF (≥17 pg/ml) were found to have a particularly poor prognosis (RR, 2.1; 95% CI, 1.2–3.6; $P = 0.0057$; Table 2).

Discussion

We found a high pretreatment S-bFGF to be associated with unfavorable survival in SCLC patients. S-bFGF was not correlated to stage or any other clinicopathological feature, except overall survival. It is of particular interest that in SCLC, S-bFGF may complement the prognostic information obtained by staging because combining the stage and S-bFGF enabled us to identify a subgroup of SCLC patients with particularly poor outcome. These results are in agreement with those we obtained in non-Hodgkin's lymphoma (16, 17) and suggest that a high S-bFGF level may reflect active angiogenesis and rapid tumor growth. Interestingly, in a recent study, Song *et al.* (19) found that elevated levels of bFGF in the conditioned medium of solid and metastatic tumors induced broad spectrum resistance to cancer drugs with diverse structures and action mechanisms (paclitaxel, doxorubicin, and 5-fluorouracil). Inhibition of bFGF by monoclonal antibody and its removal by immunoprecipitation resulted in complete reversal of the chemoresistance, and an inhibitor of bFGF (suramin) enhanced the *in vitro* and *in vivo* activity of chemotherapy, resulting in shrinkage and eradication of well-established human lung metastases in mice without enhancing toxicity. Similarly, in CLL, elevated intracellular level of bFGF is associated with resistance to chemotherapy (15).

The source of bFGF in the serum samples of our patients remains unknown. It is possible that bFGF in sera of SCLC patients is released mainly by malignant cells or by a combination of cancer cells and normal cells including the endothelial cells and peripheral blood cells. S-bFGF may alternatively be produced by normal cells under deregulated stimulation by malignant cells. Peripheral blood megakaryocytes and platelets (20), mononuclear cells (21), T cells (22, 23), macrophages (24), and granulocytes (20) have the capacity to produce bFGF. In CLL, elevated intracellular levels of bFGF are found in the CLL cells, and a high intracellular bFGF level in the CLL cells is associated with a high stage of the disease (15). In a transgenic mouse fibrosarcoma model, there is a change in the localization of bFGF from its normal cell-associated state to extracellular release in the later stages of the multistep development of fibrosarcoma. This change is concomitant with the neovascularization seen *in vivo*. Thus, in this multistep tumorigenesis pathway, there appears to be a discrete switch to the angiogenic phenotype that correlates with the export of bFGF (25). In a tumor-bearing mouse model, the origin of elevated bFGF levels in the urine was found to be almost exclusively from tumor cells (26). In agreement with this finding, i.v. administered bFGF has been found to distribute preferentially to the kidneys and the liver (27). In a study in colorectal cancer, bFGF concentrations in blood taken from mesenteric vein draining the tumor were more than 4-fold higher than those in the peripheral blood of the same patients (8). These data suggest that a large proportion of circulating bFGF in cancer patients may be derived from the tumor.

Several antiangiogenic molecules are already in preclinical and clinical testing, and their influence on angiogenic factors in serum and clinical outcome now needs to be studied. It will be of particular interest to see whether serum angiogenic factors can be used as a monitor of antiangiogenic cancer therapy. S-bFGF may be associated with outcome in many different types of human cancer in addition to lymphoma (16, 17) and SCLC because angiogenesis is required for growth and dissemination of all cancers. bFGF may also be an interesting target for antiangiogenic cancer therapy in SCLC.

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Cancer Epidemiol Biomarkers Prev 2002;11:1492-1495.

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