[CANCER RESEARCH 62, 5210–5217, September 15, 2002]

Soluble Syndecan-1 and Serum Basic Fibroblast Growth Factor Are New Prognostic Factors in Lung Cancer[1]

Heikki Joensuu,[2] Anu Anttonen, Minna Eriksson, Riitta Mäkitaro, Henrik Alfthan, Vuokko Kinnula, and Sirpa Leppä

Departments of Oncology [H. J., A. A., S. L.] and Clinical Chemistry [H. A.], Helsinki University Central Hospital, FIN-00029 Helsinki; Molecular/Cancer Biology Research Program, Biomedicum Helsinki, and Haartman Institute, FIN-00014 University of Helsinki, Helsinki [H. J., M. E., S. L.]; and Department of Internal Medicine, Oulu University Hospital, FIN-90221 Oulu [R. M., V. K.], Finland

ABSTRACT

Syndecan-1 is a ubiquitous and multifunctional extracellular matrix proteoglycan, which mediates basic fibroblast growth factor (bFGF) binding and activity. Shedding of syndecan-1 ectodomain from the plasma membrane is highly regulated. We evaluated the influence of soluble syndecan-1 and serum bFGF determined by ELISA on outcome in 184 lung cancer patients (non-small cell lung cancer, $n = 138$; small cell lung cancer, $n = 46$). Serum syndecan-1 and bFGF levels were determined from sera taken before treatment. The median follow-up of the patients alive ($n = 21$) was 8.1 years (range, 6.6–8.9 years). High serum syndecan-1 and bFGF levels tended to occur in the same patients ($P = 0.044$). When the serum values corresponding to the highest tertile were used as the cutoff value, the median survival time of the patients with a high serum syndecan-1 level (>59 ng/ml) was 4 months [95% confidence interval (CI), 3–6 months] as compared with 11 months (9–16 months) among those with lower serum levels ($P = 0.0001$), and the median survival time of the patients with a high bFGF level (>3.4 pg/ml) was 5 months (3–8 months) *versus* 11 months (8–14 months) in those with a lower level ($P = 0.023$). In general, the prognostic influence of both factors was independent of the histological subtype. Both serum syndecan-1 level (relative risk, 1.8; 95% CI, 1.1–3.1) and serum bFGF level (relative risk, 1.6; 95% CI, 1.0–2.7) had independent influence on survival in a multivariate survival analysis in non-small cell lung cancer. We conclude that high serum syndecan-1 and bFGF levels at diagnosis are associated with poor outcome in lung cancer.

INTRODUCTION

The syndecans are a family of transmembrane heparan sulfate proteoglycans, which, together with the lipid-linked glypicans, are the major source of heparan sulfates at cell surfaces (1). The syndecan family is composed of four closely related proteins (syndecan-1, syndecan-2, syndecan-3, and syndecan-4) encoded by four different genes. All adhesive cells express at least one syndecan but most express multiple syndecans (2). Syndecan-1 and syndecan-4 are expressed in a variety of cell types, including epithelial, endothelial, and vascular smooth muscle cells, syndecan-2 (fibroglycan) is expressed at high levels in cultured lung and skin fibroblasts, and syndecan-3 (N-syndecan) expression is largely restricted to central nervous system and peripheral nerves. All syndecans have an extracellular domain capable of carrying heparan sulfate and chondroitin sulfate chains, a transmembrane domain, and a cytoplasmic domain containing four universally conserved tyrosines and a conserved serine (3). Unlike syndecans, glypicans carry essentially only heparan sulfate side chains (4).

Syndecans mediate cell-cell and cell-extracellular matrix adhesion and influence cell morphology, cell growth characteristics and cell migration, signal transduction, and blood coagulation (1). These biological effects are thought to be mediated largely via the heparan sulfate chains capable of binding a variety of soluble and insoluble ligands such as growth factors, cytokines, extracellular matrix molecules, proteinases and protein inhibitors, and other biologically active molecules. By binding soluble growth factors, syndecans act as low affinity coreceptors, concentrating ligands and presenting them to the high affinity cell surface receptors (1). bFGF[3], also called FGF-2, is a prototype of heparin-binding growth factors that binds to syndecans and remains biologically active when immobilized to the cell matrix via the heparan sulfate chains of syndecan (5). The interaction between bFGF and heparan sulfate is thought to result in bFGF dimerization that facilitates growth factor binding to FGF receptor-1, which is, in turn, followed by receptor dimerization and activation of the intracellular signaling cascade (3).

bFGF is a multifunctional cytokine that has pleiotropic roles in many cell types and tissues (6). It is a major angiogenic and survival factor, it influences cell migration, and it is a cell differentiation factor involved in a variety of developmental processes. bFGF acts mainly through a paracrine/autocrine mechanism involving its cognate high affinity transmembrane receptors and heparan sulfate proteoglycan low affinity receptors, but intracellular bFGF may also have a direct biological role, particularly within the nucleus (7). Several lines of evidence support the close association between bFGF function and syndecan-1 expression and that syndecan-1 mediates bFGF binding and activity (8). A strong influence of syndecan expression on cell responsiveness to bFGF has been demonstrated in *in vitro* models, where syndecan-1 expression has resulted in a several-fold increase in cell sensitivity to bFGF (9), and syndecans have been implicated as modulators of the bFGF receptor-binding affinity resulting in decreased bFGF binding as a response to an increasing cell density in culture (10). *Syndecan-1* gene also contains an FGF-inducible response element, which is stimulated in some cells by bFGF and in others by epidermal growth factor (11).

The extracellular syndecan domains (ectodomains) can be shed from the cell surface in a process called ectodomain shedding, which includes proteolytic cleavage usually at a juxtamembrane site (12). Syndecans are constitutively shed from cultured cells as part of normal cell surface turnover (2, 13), but the syndecan-1concentrations found in sera of healthy individuals are low (14). Syndecan ectodomain shedding is a highly regulated mechanism (12) that can be accelerated by direct action of proteases such as thrombin, plasmin, and elastase (15, 16) and via receptor activation, and shedding can be inhibited by TIMP-3, a matrix-associated metalloproteinase inhibitor (12). Syndecan ectodomains can be found in fluids accumulating after injury or inflammation (16). Soluble syndecan-1 ectodomains can inhibit cell proliferation and potentially inhibit bFGF mitogenicity. As

Received 8/14/01; accepted 7/15/02.

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] This work was supported by the Academy of Finland (to H. J., S. L.), the Cancer Society of Finland (to H. J.), and Helsinki University Central Hospital research funds (to H. J., S. L.).

[2] To whom requests for reprints should be addressed, at Department of Oncology, Helsinki University Central Hospital, Haartmaninkatu 4, P. O. Box 180, FIN-00029 Helsinki, Finland. Phone: 358-9-471-73208; Fax: 358-9-471-74202; E-mail: heikki.joensuu@hus.fi.

[3] The abbreviations used are: bFGF, basic fibroblast growth factor; FGF, fibroblast growth factor; CEA, carcinoembryonal antigen; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; CI, confidence interval; RR, relative risk.

localization of syndecan-1 ectodomain, the normal cell-associated localization of bFGF may change during tumorigenesis. In a transgenic mouse fibrosarcoma model, a change from the normal cell-associated state to extracellular release of bFGF was found in the later stages of the multistep development of fibrosarcoma, which was accompanied with neovascularization seen *in vivo* (17). High serum bFGF levels have recently been found to be associated with poor outcome in non-Hodgkin's lymphoma (18) and melanoma (19).

Syndecan-1 expression as detected by immunohistochemistry is associated with poor histological grade of differentiation in squamous cell carcinomas of the head and neck and the uterine cervix, and low syndecan-1 expression in immunostaining of cancer tissue has been associated with poor outcome in head and neck cancer and mesothelioma (20–22). Soluble serum syndecan-1 has recently been found to have prognostic value in multiple myeloma (14), but to our knowledge there are no data available on the prognostic influence of soluble syndecan-1 in nonhematological cancers. Serum concentrations of syndecan-1 and bFGF have not been investigated in the same patients, although a considerable body of experimental evidence suggests that the biological effects of these two factors are closely related. In this study, we determined the serum syndecan-1 ectodomain and bFGF levels in a series of lung cancer patients and correlated the results with cancer histology because, although ubiquitous, syndecan-1 is predominantly expressed on epithelial cells in the normal tissues (23).

Table 1 *Patient and tumor characteristics*

Factor	All *n* (%)	Non-small cell lung cancer *n* (%)	Small cell lung cancer *n* (%)
Gender			
Male	158 (86)	122 (88)	36 (78)
Female	26 (14)	16 (12)	10 (22)
Karnofsky's status			
≤50	20 (11)	13 (9)	7 (15)
60	32 (17)	23 (17)	9 (20)
70	59 (32)	45 (33)	14 (30)
80	34 (18)	26 (19)	8 (17)
90–100	39 (21)	31 (22)	8 (17)
Histological type			
Small cell carcinoma	46 (25)		46 (100)
Adenocarcinoma	44 (24)	44 (32)	
Squamous cell carcinoma	82 (45)	82 (59)	
Large cell carcinoma	9 (5)	9 (7)	
Adenosquamous cell carcinoma	3 (2)	3 (2)	
Histological grade			
Grade I	7 (11)	7 (11)	
Grade II	23 (37)	23 (37)	
Grade III	32 (52)	32 (52)	
N.A.[a]	122	76	46
Stage			
I	34 (21)	28 (24)	6 (14)
II	12 (7)	10 (8)	2 (5)
IIIA	21 (13)	17 (14)	4 (9)
IIIB	36 (22)	30 (25)	6 (14)
IV	60 (37)	34 (29)	26 (59)
N.A.	21	19	2
CEA			
≤5.0 ng/ml (normal)	92	68	24
>5.0 ng/ml (>normal)	91	69	22
N.A.	1	1	0
Age at diagnosis			
Median	67	67	68
Range	(31–86)	(48–86)	(31–84)

[a] N.A., not available.

PATIENTS AND METHODS

Lung Cancer Patients. Two hundred seven patients diagnosed with lung cancer and with a serum sample taken before cancer treatment were treated in the Department of Internal Medicine, Oulu University Hospital, in 1990 to 1992. From this series, we excluded cases where no diagnostic biopsy sample was available ($n = 16$) and those with histologically unclassified lung cancer ($n = 7$), which left 184 patients with histologically diagnosed and typed lung cancer in the analysis. Histological typing of the tumors was done according to the WHO classification. The majority of the patients ($n = 138$, 75%) had NSCLC and the rest SCLC ($n = 46$, 25%). Eighty-six percent were male, and the median age was 67 (range, 31–86). The characteristics of the patients are shown in Table 1.

Fifty-one (28%) of the patients were treated with surgery consisting either of lobectomy ($n = 23$), bilateral lobectomy ($n = 6$), pulmectomy ($n = 20$), or tumorectomy ($n = 2$). Three (7%) SCLC patients underwent surgery. One hundred six patients were primarily treated either with radiation therapy ($n = 69$, 65 had NSCLC and 4 SCLC), cancer chemotherapy ($n = 13$, 5 had NSCLC and 8 SCLC), or both ($n = 24$, 4 had NSCLC and 20 SCLC). One hundred sixty-three patients had died during the follow-up, which ranged from 6.6 to 8.9 years (median, 8.1 years) for the patients still alive ($n = 21$).

Control Subjects. Serum syndecan-1 levels were measured in 100 male subjects who took part in a randomized Finnish population-based prostate cancer screening trial in 2002. The participants in this trial were identified from the Population Registry of Finland, and 69% of those who were randomized to screening based on serum prostate-specific antigen content participated (24). Serum prostate-specific antigen was first measured from the sera, and serum syndecan-1 from the frozen leftover sera stored for 1–2 months. The median age of the controls was 64 years (range, 58–71 years).

Serum Syndecan-1 Analysis. Peripheral venous blood samples were collected in sterile test tubes before any cancer therapy was given, centrifuged at $3000 \times g$ for 10 min, and then stored at −20°C. Serum syndecan-1 concentrations were determined using human syndecan-1 ELISA (Diaclone Research, Bensacon, France) according to the manufacturer's instructions. The system uses a solid phase monoclonal B-B4 antibody and a biotinylated monoclonal B-D30 antibody raised against human syndecan-1. The epitope of B-B4 has been localized between the amino acids 90 and 93 within the extracellular domain of syndecan-1 (25). The detection steps include streptavidin-horseradish peroxidase and tetramethylbenzidine as chromogens. For each analysis, 50 μl of serum were used. All analyses and calibrations were carried out in duplicate. The calibrations on each microtiter plate included recombinant human syndecan-1 standards. Optical densities were determined using a microtiter palate reader (Multiscan RC Type 351; Labsystems, Helsinki, Finland) at 450 nm. The blank was subtracted from the duplicate readings for each standard and sample. Concentrations are reported as ng/ml. No loss of syndecan-1 immunoreactivity was observed when nine samples were subjected to either three or eight freeze-thaw cycles ($P = 0.89$; Friedman test). No significant difference in serum syndecan-1 concentrations was found when cancer patient serum samples stored at −20°C for 4–6 years ($n = 22$) and samples stored for 7–10 years ($n = 66$) were compared, suggesting that there is no major loss of syndecan-1 immunoreactivity during long-term storage as compared with shorter storage ($P = 0.18$; the Mann-Whitney test, data not shown).

Serum bFGF Analysis. Serum bFGF concentrations were determined as serum bFGF immunoreactivity using a quantitative sandwich enzyme immunoassay technique (Quantikine High Sensitivity Human FGF Basic Immunoassay; R&D Systems) as described earlier (26). The system uses a solid phase monoclonal and an enzyme-linked polyclonal antibody raised against recombinant human bFGF. The analysis was performed essentially as the syndecan-1 immunoassay. Absorbances were determined at 490 nm. Serum syndecan-1 levels were determined without any knowledge of the survival or other clinical data. We found no association between serum bFGF concentrations and the duration of storage in serum samples stored at −20°C for 11–17 years (26).

Immunohistochemistry for Syndecan-1 Expression. The analysis of syndecan-1 expression was made from formalin-fixed and paraffin-embedded tumor samples by immunohistochemistry. Tissue samples were cut into 5-μm sections on Vectabond slides. The slides were kept at 37°C no longer than for 24 h and after that deparaffinized and dehydrated. Syndecan-1 was localized using a monoclonal B-B4 antibody (Serotec, Oxford, United Kingdom) and the avidin-biotin immunoperoxidase method. Pretreated slides were incubated with 2% normal goat serum in 1% BSA (Sigma, St. Louis, MO) for 20 min at room temperature, followed by B-B4 antibody in 0.3% BSA overnight at room temperature. After washing twice with PBS, the slides were incubated with Vectastain biotinylated antimouse IgG (Vector Laboratories, Burlingame, CA) in 0.3% BSA for 30 min at room temperature, then washed twice with PBS, followed by avidin DH-biotinylated horseradish peroxidase mixture according to the manufacturer's instructions (Vector Laboratories) for 1 h at room

temperature. After incubation, the slides were washed again twice with PBS, and for color reaction, the slides were incubated with 0.02% 3-amino-9-ethylcarbazole (in *N,N*-dimethylformamide) and 0.1% hydrogen peroxidase in 0.05 M acetate buffer (pH 5.0) for 20 min at room temperature, counterstained with hematoxylin, and mounted.

Immunoreactivity for syndecan-1 was classified by assessing visually the percentage of syndecan-1-positive tumor cells of all tumor cells in the field. First, the percentage of syndecan-1-positive tumor cells of all tumor cells was calculated from three representative microscope fields/slide (Olympus Optical Company, Tokyo, Japan; ×10 objective, diameter 2.6 mm, area 5.3 mm^2). All selected fields contained at least 100 cancer cells, and necrotic areas and those with marked inflammation were excluded. Assessment of syndecan expression was done blindly without any knowledge of the clinical or survival data.

Serum CEA. CEA was quantitated in serum by an immunofluorometric assay (AutoDELFIA; Wallac, Turku, Finland). The detection limit of the assay is 0.2 ng/ml. The upper reference limit is 5 ng/ml.

Statistical Analysis. Statistical analyses were done using a BMDP computer program (BMDP Statistical Software; University of California Press, Los Angeles, CA). Cumulative survival was estimated with the product limit method. The log-rank test was used for comparison of survival between groups. The Brookmeyer-Crowley CIs were computed for the median survival times. Frequency tables were analyzed with the χ^2 test. Comparison of non-normal distributions was done by computing the Spearman's correlation coefficient, and nonnormally distributed parameters between two groups were compared with the Mann-Whitney test. The relative importance of prognostic factors was analyzed with Cox's proportional hazard's regression analysis (BMDP 2L). All *P*s are two-tailed.

RESULTS

Serum Syndecan-1 and bFGF Levels in NSCLC and in SCLC. The median serum syndecan-1 level was 40 ng/ml (range, 7–414 ng/ml) in patients with NSCLC and 44 ng/ml (range, 11–385 ng/ml) in those with SCLC ($P = 0.57$). The levels measured in lung cancer patients were higher than those found in the controls, who had a median serum level of 16 ng/ml (range, from undetectable to 213 ng/ml; $P < 0.0001$, Fig. 1). The difference between lung cancer patients and controls persisted when the female patients ($n = 26$) were excluded from the analysis ($P < 0.0001$). Ninety-five percent of the controls had serum syndecan-1 level 61 ng/ml or lower.

Similarly, there was no significant difference in the serum bFGF levels between patients with NSCLC and those with SCLC (median, 4.2 pg/ml; range, from undetectable to 62.6 pg/ml *versus* median, 1.8 pg/ml; range, from undetectable to 8.6 pg/ml, respectively; $P = 0.32$). These serum bFGF levels are similar as have been found earlier in sera of patients with non-Hodgkin's lymphoma or melanoma (19, 20). High serum syndecan-1 and bFGF levels tended to occur in the same patients in the entire series (Spearman correlation coefficient $P = 0.044$; $n = 184$).

Association of Serum Syndecan-1 Levels with Survival in Univariate Analyses. High serum syndecan-1 levels were generally associated with poor outcome. When the median (41 ng/ml) of the entire series ($n = 184$) was taken as the cutoff value, patients with higher than the median serum syndecan-1 level had a median survival time of 6 months (Brookmeyer-Crowley 95% CI from 4 to 9 months) as compared with 11 months (8–17 months) in patients who had serum levels lower than the median ($P = 0.0030$; Fig. 2*A*). When the serum syndecan-1 level corresponding to the highest tertile (59 ng/ml) was used as the cutoff level instead of the median value, the median survival time of the patients with a high serum level turned out to be only 4 months (3- 6 months) as compared with 11 months (9–16 months) among the rest of the patients ($P = 0.0001$; Fig. 2*B*). These findings remained essentially similar when the median serum syndecan-1 level or the level corresponding to the upper tertile were used as cutoff values among the subset of patients with NCSLC or among those with SCLC (Fig. 3; Table 2). Similarly, patients with adenocarcinoma ($n = 44$) and those with squamous cell carcinoma ($n = 82$) and with a high serum syndecan-1 level had generally poor outcome (Fig. 4).

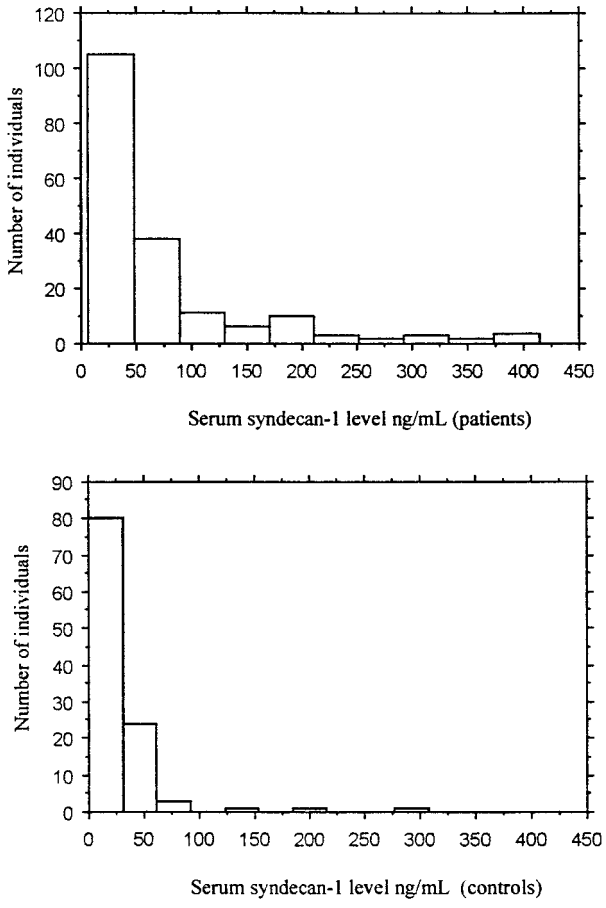

Fig. 1. Serum syndecan-1 levels at diagnosis in 184 lung cancer patients and 100 controls representing the normal male population with a similar median age.

To investigate the association between serum syndecan-1 level and outcome in patients with different tumor stage at the time of the diagnosis, we examined the prognostic value of serum syndecan-1 level separately among patients with a more limited NSCLC (stages I to IIIA, $n = 55$) and among those with a more advanced NSCLC at diagnosis (stages IIIB and IV, $n = 64$). In stages I to IIIA, patients with lower than the median serum syndecan-1 concentration tended to have a longer median survival time than those with a higher than the median serum syndecan-1 level (40 *versus* 25 months, respectively; $P = 0.070$), and the same was found in stage IIIB or IV cancer (5 *versus* 3 months, respectively; $P = 0.029$), suggesting that high serum syndecan-1 levels are associated with poor outcome both among patients with limited NSCLC and among those with a more advanced disease.

Association of Serum bFGF Levels with Survival in Univariate Analyses. As serum syndecan-1 levels, high serum bFGF levels were also associated with a poor outcome. In general, patients who had lower than the median serum bFGF levels had ~2-fold longer median survival times than those with serum levels above the median; the difference became more marked when the upper tertile was used as the cutoff value instead of the median serum value (Fig. 5; Table 3).

When the association of serum bFGF with survival was investigated in the subset of smaller NSCLC stages (stage I to IIIA, $n = 38$), no difference in survival was found between those with lower or

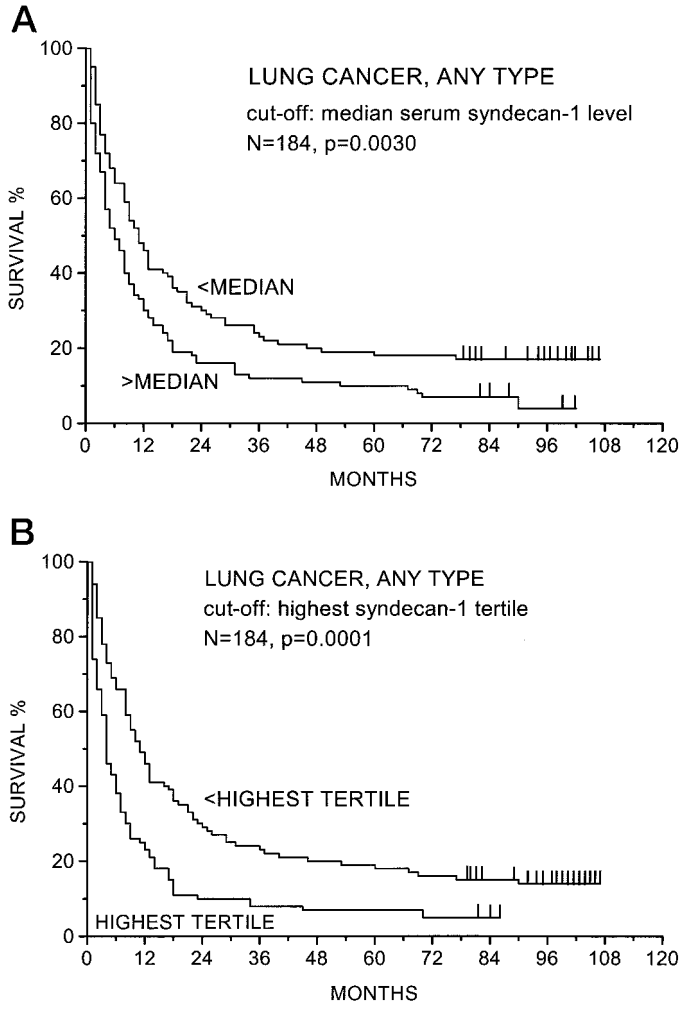

Fig. 2. Influence of serum syndecan-1 levels on survival in lung cancer. *A*, the median serum level (41 ng/ml) was used as the cutoff value; *B*, the value corresponding to the highest tertile (59 ng/ml) was used as the cutoff value. Patients alive at the time of the analysis are indicated by a *bar*.

higher than the median serum bFGF levels ($P = 0.51$). However, patients with higher than the median bFGF levels turned out to have shorter survival than those with lower serum levels when only patients with stage IIIB or IV NSCLC were included in the analysis (median survival, 3 months and 95% CI, 2–4 months *versus* median survival, 6 months and 95% CI, 5–16 months, respectively; $P = 0.0034$).

Association of Serum Syndecan-1 and bFGF Levels with Clinicopathological Parameters. Patients with a poor performance status (Karnofsky score ≤70) and those with a large tumor mass (stage IIIB or stage IV disease) had high serum syndecan-1 levels ($P = 0.0011$ and $P = 0.0004$, respectively; Table 4; Fig. 6). No such association was found between the serum syndecan-1 level and gender, age at diagnosis (tested ≤median *versus* >median), histological grade (well or moderately well differentiated *versus* poorly differentiated), histological type (NSCLC *versus* SCLC or adenocarcinoma *versus* squamous cell carcinoma), or serum CEA level at diagnosis (tested ≤5 ng/ml (normal) *versus* >5 ng/ml, ≤3.9 ng/ml (median) *versus* > 3.9 ng/ml, ≤5.7 ng/ml (the upper tertile) *versus* >5.7 ng/ml), when either the median serum syndecan-1 level (41 ng/ml) or the level corresponding to the highest tertile (59 ng/ml) was used as the cutoff value ($P > 0.05$ in all analyses). Although we found a weak positive association between the serum bFGF and syndecan-1 levels when tested using the Spearman correlation coefficient ($P = 0.044$), no significant association between these factors was found when the median levels or the levels corresponding to the highest tertiles were used as the cutoff values (Table 4), suggesting that the positive association between serum syndecan-1 and bFGF levels is weak in patients with lung cancer.

When the serum bFGF level corresponding to the highest tertile (3.4 pg/ml) was used as the cutoff value, patients with a poor performance status (tested Karnofsky score ≤70 *versus* >70) and those with NSCLC (tested NSCLC *versus* SCLC) tended to have high serum levels ($P = 0.050$ $P =$ and 0.054, respectively; Table 4). NSCLC patients with adenocarcinoma had higher levels than those with squamous cell cancer ($P = 0.027$). Gender, age at diagnosis (tested ≤median *versus* >median), stage (I to IIIA *versus* IIIB or IV), serum CEA levels, or the histological grade (well or moderately well differentiated *versus* poorly differentiated) were not associated with higher than the median serum bFGF levels or levels within the highest tertile ($P > 0.05$ for all analyses).

Association of Serum Syndecan-1 and bFGF Levels with Syndecan-1 Expression on Cancer Cells in Histological Biopsies. To study whether serum syndecan-1 and bFGF levels are associated with expression of syndecan-1 on cancer cells, the available histological biopsies taken from the tumors before cancer treatment ($n = 45$) were immunostained with an antisyndecan-1 antibody, and the results were correlated with the serum syndecan-1 and bFGF levels. Synde-

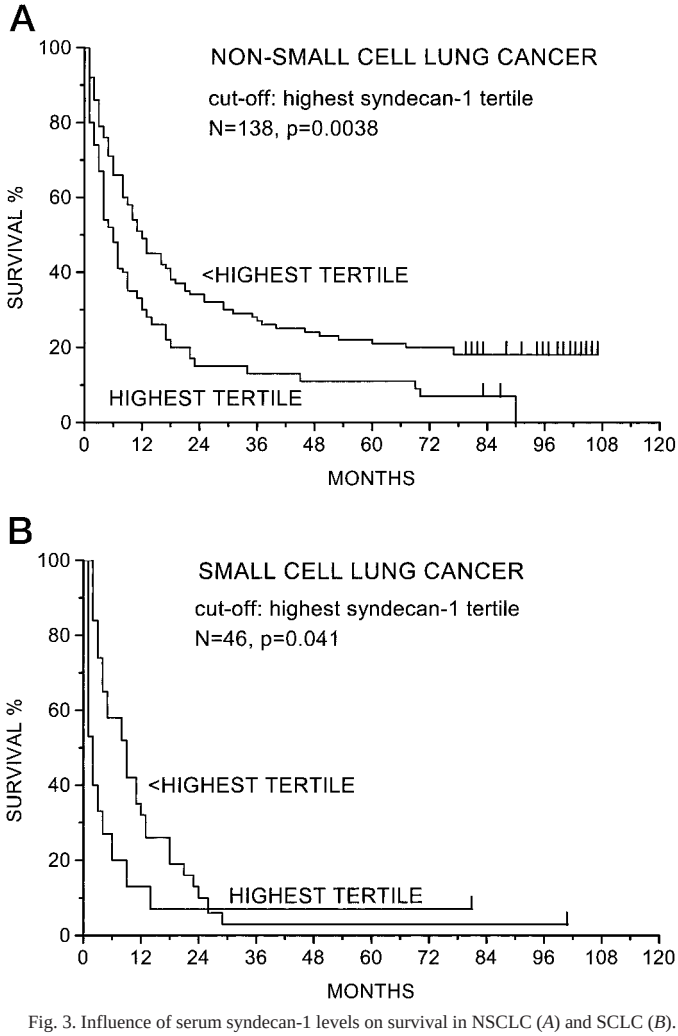

Fig. 3. Influence of serum syndecan-1 levels on survival in NSCLC (*A*) and SCLC (*B*). The serum levels corresponding to the highest tertile (54 ng/ml, *A*; 84 ng/ml, *B*) were used as the cutoff values. Patients alive at the time of the analysis are indicated by a *bar*.

Table 2 *Association of serum syndecan-1 level with overall survival in univariate analyses*

Histology	*n*	Median survival mo (95% CI)	1-yr survival, %	5-yr survival, %	*P*
Lung cancer, all					
≤41 ng/ml (median)	94	11 (8–17)	48	18	
>41 ng/ml	90	6 (4–9)	33	10	0.0030
≤59 ng/ml (upper tertile)	123	11 (9–16)	49	19	
>59 ng/ml	61	4 (3–6)	25	7	0.0001
NSCLC					
≤40 ng/ml (median)	69	13 (9–21)	54	23	
>40 ng/ml	69	7 (4–10)	36	13	0.0085
≤54 ng/ml (upper tertile)	92	12 (8–18)	51	22	
>54 ng/ml	46	6 (4–9)	33	11	0.0038
NSCLC, squamous cell carcinoma					
≤40 ng/ml (median)	43	13 (10–22)	58	23	0.11
>40 mg/ml	39	8 (4–18)	44	18	
≤54 ng/ml (upper tertile)	55	13 (10–25)	58	24	0.029
>54 ng/ml	27	8 (4–14)	37	15	
NSCLC, adenocarcinoma					
≤38 mg/ml (median)	23	11 (6–35)	48	22	0.0067
>38 ng/ml	21	4 (1–7)	19	5	
≤53 ng/ml (upper tertile)	30	9 (5–21)	43	20	0.0021
>53 ng/ml	14	4 (1–7)	14	0	
SCLC					
≤44 ng/ml (median)	23	8 (3–11)	30	4	
>44 ng/ml	23	4 (2–9)	26	4	0.29
≤84 ng/ml (upper tertile)	31	9 (4–12)	35	3	
>84 ng/ml	15	2 (1–4)	13	7	0.041

can-1 expression varied in the tissue biopsies from entirely absent ($n = 11$) to weakly positive (+, $n = 11$), moderately positive (++, $n = 13$), and strongly positive (+++, $n = 10$). Despite the cutoff level chosen for syndecan-1 expression (0 *versus* >0, 0 or + *versus* ++ or +++, or 0 to ++ *versus* +++), serum syndecan-1 levels did not have significant association with cancer cell syndecan-1 expression ($P > 0.3$ for all analyses; the Mann-Whitney test). Patients who had cancer with low syndecan-1 expression (0 or +) tended to have lower serum bFGF levels than those with stronger expression (++ or +++; median, 1.2 pg/ml; range, undetectable to 5.0 pg/ml *versus* median, 3.6 pg/ml; range, 0.5–8.9 pg/ml, respectively; $P = 0.018$). However, such a significant association was not found when other cutoff levels for tissue syndecan-1 expression were tested ($P > 0.4$ for both analyses). Syndecan-1 expression as detected by immunohistochemistry was not associated with survival regardless of the degree of expression chosen as the cutoff value ($P > 0.10$ for all analyses).

Multivariate Survival Analyses. To find out whether serum syndecan-1 and bFGF have independent influence on survival in NSCLC, factors that were significantly ($P < 0.05$) associated with survival in a univariate survival analysis (stage, Karnofsky's performance score, and serum CEA, syndecan-1 and bFGF levels) were entered into Cox's stepwise multivariate model. Both serum syndecan-1 level (RR, 1.8; 95% CI, 1.1–3.1) and serum bFGF level (RR, 1.6; 95% CI, 1.0–2.7) had independent influence on survival as well as stage at diagnosis (RR, 2.8; 95% CI, 1.7–4.8), whereas Karnofsky's status and serum CEA level did not (Table 5).

A similar multivariate analysis was performed within the subset of SCLC patients. Serum syndecan-1 level, age at diagnosis, Karnofsky's performance score, and stage at diagnosis were entered into Cox's stepwise multivariate analysis as covariables, whereas serum bFGF and CEA levels were not because they were not significantly associated with survival in a univariate survival analysis (Table 3). Stage at diagnosis (RR, 3.4; 95% CI, 1.6–2.1) and Karnofsky's performance status (RR, 3.2; 95% CI, 1.6–6.8) were significantly associated with survival in the multivariate analysis, and serum syndecan-1 level had possibly independent influence on survival (RR, 1.9; 95% CI, 0.9–2.1; $P = 0.08$; Table 5).

DISCUSSION

In this study, both serum syndecan-1 and bFGF levels were associated with overall survival in univariate analyses, both factors had independent influence on survival in a multivariate analysis in NSCLC, and soluble syndecan-1 possibly also in SCLC. The effects were largely independent of cancer histological type and generally similar irrespective of whether the medians or the serum levels corresponding to the highest tertiles were used as the cutoff values, although the cutoff values for the highest tertiles tended to produce a larger survival difference between the high and the low serum value groups. Ninety-five percent of the controls had serum syndecan-1 level 61 ng/ml or lower, which roughly corresponds to the cutoff for the higher tertile in cancer patients (59 ng/ml). These findings suggest that serum syndecan-1 and serum bFGF are powerful prognostic factors in lung cancer.

Squamous cell carcinomas commonly express syndecan-1 (20, 21, 23, 27). The majority of NSCLCs also express syndecan-1 in immunohistochemistry of tumor tissue (76% in the present series and 72 and 87% in Refs. 27, 28, respectively). There is paucity of data on syndecan expression in human adenocarcinomas, but pancreatic cancer cells express syndecan-1 in moderate to high levels in the majority of the cancer cells unlike esophageal, gastric, colon, and liver adenocarcinomas, and pancreatic cancer cell lines release syndecan-1 into

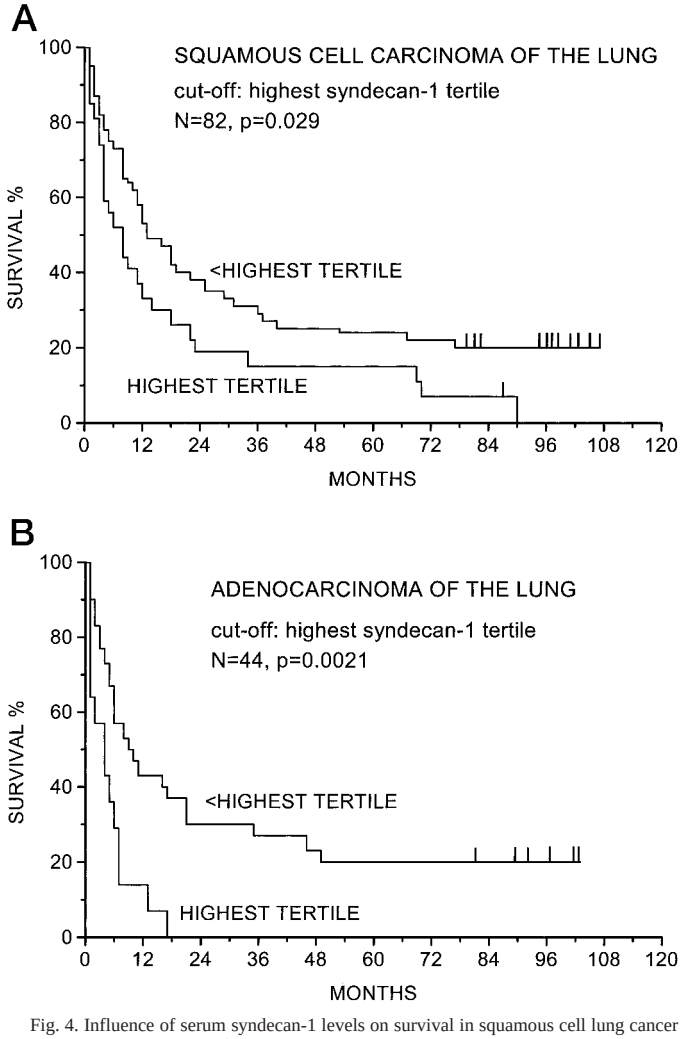

Fig. 4. Influence of serum syndecan-1 levels on survival in squamous cell lung cancer (*A*) and in adenocarcinoma of the lung (*B*). The serum levels corresponding to the highest tertile (54 ng/ml, *A*; 38 ng/ml, *B*) were used as the cutoff values. Patients alive at the time of the analysis are indicated by a *bar*.

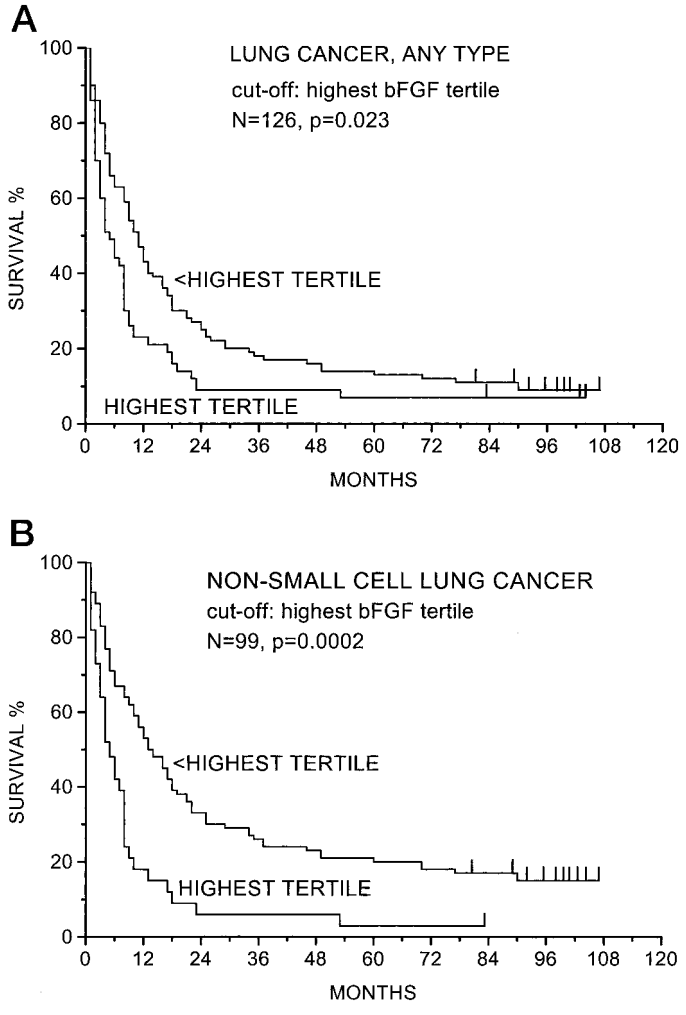

Fig. 5. Influence of serum bFGF levels on survival in lung cancer (*A*) and in NSCLC (*B*). The serum levels corresponding to the highest tertile (3.4 pg/ml, *A*; 4.1 pg/ml, *B*) were used as the cutoff values. Patients alive at the time of the analysis are indicated by a *bar*.

the culture medium (29). The prognostic role of serum soluble syndecan-1 now needs to be further evaluated not only in human squamous cell cancers but in some adenocarcinomas as well.

High cancer cell syndecan-1 expression in tissue biopsies has been found to be associated with favorable outcome in head and neck cancer (20, 21), squamous cell lung cancer (27), and mesothelioma (22), and loss of syndecan-1 expression is associated with poor histological grade of differentiation in squamous cell lung carcinoma (27), increasing aggressiveness of basal cell carcinoma (30), and with low grade of differentiation and presence of metastases in hepatocellular carcinoma (31). Hypothetically, the extracellular syndecan-1 domain might be shed more often by proteolytic cleavage in aggressive tumors than in the more indolent ones leading to higher serum concentrations in the former. We found, however, no significant association between the syndecan-1 tumor tissue expression and the serum levels, but this finding should be interpreted with caution because we had adequate tissue samples only from 45 patients available for syndecan-1 immunostaining. High levels of circulating syndecan-1 may also in part reflect the presence of a large tumor mass. In the present series, serum syndecan-1 levels $>$ 59 ng/ml were found in as many as 63% of the patients with stage IIIB or IV disease at presentation as compared with only 16% of those with stage I to IIIA disease ($P = 0.0004$; Table 4).

A weak positive association was found between serum bFGF and syndecan-1 levels. However, both factors had independent prognostic influence in NSCLC in a multivariate model, suggesting that although syndecan expression enhances the biological activity of bFGF and facilitates its binding to the high affinity receptors, many of the biological effects of bFGF may be independent of the amount of syndecan-1 ectodomain shed from cancer tissue. Interestingly, serum bFGF levels were neither associated with the histological grade nor cancer stage at diagnosis. bFGF is a potent angiogenesis promoting growth factor, which might explain the association of bFGF with poor outcome. Known up-regulators of bFGF include hypoxia (32) and transforming growth factor β1 (33), and the origin of elevated urine bFGF levels has been found to be almost solely from tumor cells in a mouse model (34).

Nine NSCLC patients in this series had been treated with cancer chemotherapy. Hypothetically, systemic cancer chemotherapy may be a confounding factor influencing the prognostic significance of serum syndecan-1 or that of serum bFGF. Chemotherapy is commonly given an adjuvant treatment or concurrently with radiation therapy as the primary therapy of lung cancer, and it is increasingly difficult to find modern lung cancer series where chemotherapy has not been used. However, when the nine patients with NSCLC who had been given cancer chemotherapy at the time of the diagnosis are excluded from the analysis, serum syndecan-1 level is still strongly associated with survival irrespective of whether the median or the highest tertile is used as the cutoff value (the median survival time was 12 months for patients with lower than the median serum syndecan-1 level, and 7 months for those with a higher level, $P = 0.010$; for patients with lower level than the value corresponding to the highest tertile 12 months, and 6 months for those with serum syndecan-1 within the highest tertile, $P = 0.0052$). Similarly, patients with serum bFGF within the highest tertile still have inferior outcome when the few patients treated with chemotherapy are excluded from the series ($P = 0.0061$). Soluble syndecan-1 may be of particular interest as a predictive factor for treatment efficacy in patients treated with matrix proteinase inhibitors and serum bFGF in patients treated with novel antiangiogenic agents.

Table 3 *Association of serum bFGF level with overall survival in univariate analyses*

Histology	*n*	Median survival, mo (95% CI)	1-yr survival, %	5-yr survival, %	*P*
Lung cancer, all					
≤2.2 pg/ml (median)	65	11 (8–16)	46	14	
>2.2 pg/ml	61	6 (4–8)	31	10	0.16
≤3.4 pg/ml (upper tertile)	86	11 (8–14)	47	14	
>3.4 pg/ml	43	5 (3–8)	23	7	0.023
NSCLC					
≤2.3 pg/ml (median)	51	13 (8–18)	53	20	
>2.3 pg/ml	48	6 (4–9)	33	10	0.082
≤4.1 pg/ml (upper tertile)	66	13 (10–19)	56	21	
>4.1 pg/ml	33	5 (3–8)	18	3	0.0002
NSCLC, squamous cell carcinoma					
≤2.1 pg/ml (median)	31	12 (8–18)	52	19	0.45
>2.1 pg/ml	30	8 (4–19)	43	13	
≤2.8 pg/ml (upper tertile)	41	12 (8–18)	54	22	0.15
>2.8 pg/ml	20	8 (4–19)	35	5	
NSCLC, adenocarcinoma					
≤4.1 pg/ml (median)	15	17 (5–46)	60	20	0.0031
>4.1 pg/ml	15	5 (2–8)	13	0	
≤5.1 pg/ml (upper tertile)	21	6 (4–21)	43	14	0.12
>5.1 pg/ml	9	6 (1–10)	22	0	
SCLC					
≤1.8 pg/ml (median)	14	9 (3–12)	29	0	
>1.8 pg/ml	13	3 (2–9)	15	0	0.74
≤2.9 pg/ml (upper tertile)	18	8 (3–11)	28	0	
>2.9 pg/ml	9	2 (−)	11	0	0.67

Table 4 *Association between the serum syndecan-1 and bFGF level and the Karnofsky's performance status, clinical stage, and the histological type*

	Serum syndecan-1 (ng/ml)		Serum bFGF (pg/ml)	
	≤59 (upper tertile)	>59	≤3.4 (upper tertile)	>3.4
Factor	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Karnofsky's status				
≤70	64 (58)	47 (42)	47 (60)	32 (41)
>70	59 (81)	14 (19)	36 (77)	11 (23)
	$P = 0.0011$		$P = 0.050$	
Stage				
I, II, or IIIA	56 (84)	11 (16)	35 (76)	11 (34)
IIIB or IV	55 (57)	41 (63)	40 (62)	25 (39)
	$P = 0.0004$		$P = 0.11$	
Histological type				
NSCLC	95 (69)	43 (31)	61 (62)	38 (38)
SCLC	28 (61)	18 (39)	22 (82)	5 (18)
	$P = 0.32$		$P = 0.054$	
Adenocarcinoma	31 (71)	13 (30)	14 (47)	16 (53)
Squamous cell carcinoma	58 (71)	24 (29)	43 (71)	18 (30)
	$P = 0.97$		$P = 0.027$	
Serum bFGF level				
≤2.2 pg/ml (median)	48 (74)	17 (26)		
>2.2 pg/ml	42 (69)	19 (31)		
	$P = 0.54$			
≤3.4 pg/ml (upper tertile)	63 (76)	20 (24)		
>3.4 pg/ml	27 (63)	16 (37)		
	$P = 0.12$			

Established adverse prognostic factors in NSCLC and SCLC consist of advanced stage at diagnosis, ages > 60 years at diagnosis, male gender, and a poor performance status and weight loss, but many other factors have been investigated with variable success (35–43). Of the established prognostic factors, a high serum syndecan-1 level was significantly associated with an advanced stage and a poor performance status, and a high serum bFGF level was associated with a poor performance status. These two serum factors are quick to measure with ELISA and relatively inexpensive and might be incorporated into a panel of prognostic factors in lung cancer. However, confirmatory studies in other series of patients need to be carried out before recommending their use in the clinical decision-making.

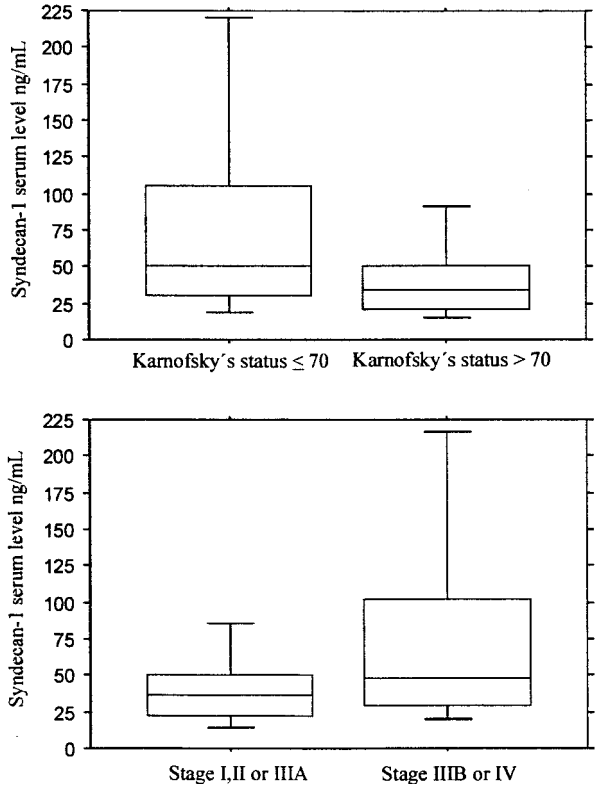

Fig. 6. *Box plots* illustrating the associations between serum syndecan-1 levels at diagnosis and the Karnofsky's performance status (*A*) and the stage of disease (*B*).

Table 5 *Results of Cox's stepwise proportional hazard model in NSCLC and SCLC*

Factor	β	SE(β)	*P*	RR of death (95% CI)
NSCLC				
Stage				
IV *versus* III *versus* II or I	1.036	0.270	0.001	2.8 (1.7–4.8)
Serum syndecan-1 level				
Highest tertile *versus* less	0.588	0.271	0.011	1.8 (1.1–3.1)
Serum bFGF level				
Highest tertile *versus* less	0.497	0.255	0.056	1.6 (1.0–2.7)
Karnofsky's status			N.S.[a]	
≤70 *versus* >70				
Serum CEA			N.S.	
Normal *versus* > normal				
SCLC				
Stage				
IV *versus* III or II or I	1.214	0.387	0.001	3.4 (1.6–2.1)
Karnofsky's status				
≤70 *versus* >70	1.178	0.374	0.002	3.2 (1.6–6.8)
Serum syndecan-1 level				
Highest tertile *versus* less	0.663	0.372	0.083	1.9 (0.9–2.1)
Age at diagnosis			N.S.	
>68 (median)				
≤68				

[a] N.S., not significant.

We conclude that high serum soluble syndecan-1 level is associated with poor outcome in NSCLC and SCLC and that a high serum bFGF level is associated with poor survival in NSCLC. A weak positive association exists between the serum levels of these factors. Elevated levels of syndecan-1 and bFGF are more common in patients who have a poor performance status, and high serum syndecan-1 levels are associated with advanced cancer. Serum syndecan-1 levels have prognostic influence also in adenocarcinoma of the lung. Soluble syndecan-1 and bFGF now need to be studied as prognostic and predictive factors for treatment efficacy in various histological types of human cancer, and they might be of particular interest as potential factors predicting for treatment response to matrix metalloproteinase inhibitors or to antiangiogenic drugs.

REFERENCES

1. Bernfield, M., Götte, M., Park, P. W., Reizes, O., Fizgerald, M. L., Lincecum, J., and Zako, M. Functions of cell surface heparan sulfate proteoglycans. Annu. Rev. Biochem., *68:* 729–777, 1999.
2. Kim, C. W., Goldberger, O. A., Gallo, R. L., and Bernfield, M. Members of the syndecan family of heparan sulfate proteoglycans are expressed in distinct cell-, tissue-, and development-specific patterns. Mol. Biol. Cell, *5:* 797–805, 1994.
3. Volk, R., Schwartz, J. J., Li, J., Rosenberg, R. D., and Simons, M. The role of syndecan cytoplasmic domain in basic fibroblast growth factor-dependent signal transduction. J. Biol. Chem., *274:* 24417–24424, 1999.
4. Aviezer, D., Levy, E., Safran, M., Svahn, C., Buddecke, E., Schmidt, A., David, G., Vlodavsky, I., and Yayon, A. Differential structural requirements of heparin and heparan sulfate proteoglycans that promote binding of basic fibroblast growth factor to its receptor. J. Biol. Chem., *269:* 114–121, 1994.
5. Salmivirta, M., Heino, J., and Jalkanen, M. Basic fibroblast growth factor-syndecan complex at cell surface or immobilized to matrix promotes cell growth. J. Biol. Chem., *267:* 17606–17610, 1992.
6. Okada-Ban, M., Thiery, J. P., and Jouanneau, J. Fibroblast growth factor-2. Int. J. Biochem. Cell Biol., *32:* 263–267, 2000.
7. Nugent, M. A., and Iozzo, R. V. Fibroblast growth factor-2. Int. J. Biochem. Cell Biol., *32:* 115–120, 2000.
8. Filla, M. S., Dam, P., and Rapraeger, A. C. The cell surface proteoglycan syndecan-1 mediates fibroblast growth factor-2 binding and activity. J. Cell. Physiol., *174:* 310–321, 1998.
9. Larrain, J., Carey, D. J., and Brandan, E. Syndecan-1 expression inhibits myoblast differentiation through a basic fibroblast growth factor-dependent mechanism. J. Biol. Chem., *273:* 32288–32296, 1998.
10. Richardson, T. P., Trinkaus-Randall, V., and Nugent, M. A. Regulation of basic fibroblast growth factor binding and activity by cell density and heparan sulfate. J. Biol. Chem., *274:* 13534–13540, 1999.
11. Jaakkola, P., Maatta, A., and Jalkanen, M. The activation and composition of FiRE (an FGF-inducible response element) differ in a cell type- and growth factor-specific manner. Oncogene, *17:* 1279–1286, 1998.
12. Fitzgerald, M. L., Wang, Z., Park, P. W., Murphy, G., and Bernfield, M. Shedding of syndecan-1 and -4 ectodomains is regulated by multiple signaling pathways and mediated by a TIMP-3-sensitive metaloproteinase. J. Cell Biol., *148:* 811–824, 2000.
13. Spring, J., Paine-Saunders, S. E., Hynes, R. O., and Bernfield, M. Drosophila syndecan: conservation of a cell surface heparan sulfate proteoglycan. Proc. Natl. Acad. Sci. USA, *91:* 3334–3338, 1994.
14. Seidel, C., Sundan, A., Hjorth, M., Turesson, I., Dahl, I. M., Abildgaard, N., Waage, A., and Borset, M. Serum syndecan-1: a new independent prognostic marker in multiple myeloma. Blood, *95:* 388–392, 2000.
15. Buczek-Thomas, J. A., and Nugent, M. A. Elastase-mediated release of heparan sulfate proteoglycans from pulmonary fibroblast cultures. A mechanism for basic fibroblast growth factor (bFGF) release and attenuation of bfgf binding following elastase-induced injury. J. Biol. Chem., *274:* 25167–25172, 1999.
16. Subramanian, S. V., Fitzgerald, M. L., and Bernfield, M. Regulated shedding of syndecan-1 and -4 ectodomains by thrombin and growth factor receptor activation. J. Biol. Chem., *272:* 14713–14720, 1997.
17. Kandel, J., Bossy-Wetzel, E., Radvanyi, F., Klagsbrun, M., Folkman, J., and Hanahan, D. Neovascularization is associated with a switch to the export of bFGF in the multistep development of fibrosarcoma. Cell, *66:* 1095–1104, 1991.
18. Salven, P., Orpana, A., Teerenhovi, L., and Joensuu, H. Simultaneous elevation in the serum concentrations of the angiogenic growth factors VEGF and bFGF is an independent predictor of poor prognosis in non-Hodgkin lymphoma: a single-institution study of 200 patients. Blood, *96:* 3712–3718, 2000.
19. Ugurel, S., Rappl, G., Tilgen, W., and Reinhold, U. Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. J. Clin. Oncol., *19:* 577–583, 2001.
20. Anttonen, A., Kajanti, M., Heikkila, P., Jalkanen, M., and Joensuu, H. Syndecan-1 expression has prognostic significance in head and neck carcinoma. Br. J. Cancer, *79:* 558–564, 1999.
21. Pulkkinen, J. O., Penttinen, M., Jalkanen, M., Klemi, P., and Grenman, R. Syndecan-1: a new prognostic marker in laryngeal cancer. Acta Oto-Laryngol., *117:* 312–315, 1997.
22. Kumar-Singh, S., Jacobs, W., Dhaene, K., Weyn, B., Bogers, J., Weyler, J., and Van Marck, E. Syndecan-1 expression in malignant mesothelioma: correlation with cell differentiation, WT1 expression, and clinical outcome. J. Pathol., *186:* 300–305, 1998.
23. Bayer-Garner, I. B., Sanderson, R. D., and Smoller, B. R. Syndecan-1 expression is diminished in acantholytic cutaneous squamous cell carcinoma. J. Cutan. Pathol., *26:* 386–390, 1999.
24. Maattanen, L., Auvinen, A., Stenman, U. H., Tammela, T., Rannikko, S., Aro, J., Juusela, H., and Hakama, M. Three-year results of the Finnish prostate cancer screening trial. J. Natl. Cancer Inst. (Bethesda), *93:* 552–553, 2001.
25. Dore, J. M., Morard, F., Vita, N., and Wijdenes, J. Identification and location on syndecan-1 core protein epitopes of B-B2 and B-B4 monoclonal antibodies. FEBS Lett., *426:* 67–70, 1998.
26. Salven, P., Teerenhovi, L., and Joensuu, H. A high pretreatment serum bFGF concentration is an independent predictor of poor prognosis in non-Hodgkin's lymphoma. Blood, *94:* 3334–3339, 1999.
27. Anttonen, A., Heikkila, P., Kajanti, M., Jalkanen, M., and Joensuu, H. High syndecan-1 expression is associated with favourable outcome in squamous cell lung carcinoma treated with radical surgery. Lung Cancer, *32:* 297–305, 2001.
28. Toyoshima, E., Ohsaki, Y., Nishigaki, Y., Fujimoto, Y., Kohgo, Y., and Kikucki, K. Expression of syndecan-1 is common in human lung cancers independent of expression of epidermal growth factor receptor. Lung Cancer, *31:* 193–202, 2001.
29. Conejo, J. R., Kleeff, J., Koliopanos, A., Matsuda, K., Zhu, Z. W., Goecke, H., Bicheng, N., Zimmermann, A., Kore, M., Friess, H., and Buchler, M. W. Syndecan-1 expression is up-regulated in pancreatic but not in other gastrointestinal cancers. Int. J. Cancer, *88:* 12–20, 2000.
30. Bayer-Garner, I. B., Dilday, B., Sanderson, R. D., and Smoller, B. R. Syndecan-1 expression is decreased with increasing aggressiveness of basal cell carcinoma. Am. J. Dermatopathol., *22:* 119–122, 2000.
31. Matsumoto, A., Ono, M., Fujimoto, Y., Gallo, R. L., Bernfield, M., and Kohgo, Y. Reduced expression of syndecan-1 in human hepatocellular carcinoma with high metastatic potential. Int. J. Cancer, *74:* 482–491, 1997.
32. Kuwabara, K., Ogawa, S., Matsumoto, M., Koga, S., Clauss, M., Pinsky, D. J., Lyn, P., Leavy, J., Witte, L., Joseph-Silverstein, J., *et al*. Hypoxia-mediated induction of acidic/basic fibroblast growth factor and platelet-derived growth factor in mononuclear phagocytes stimulates growth of hypoxic endothelial cells. Proc. Natl. Acad. Sci. USA, *92:* 4606–4610, 1995.
33. McCartney-Francis, N., Mizel, D., Wong, H., Wahl, L., and Wahl, S. TGF-β regulates production of growth factors and TGF-β by human peripheral blood monocytes. Growth Factors, *4:* 27–35, 1990.
34. Soutter, A. D., Nguyen, M., Watanabe, H., and Folkman, J. Basic fibroblast growth factor secreted by an animal tumor is detectable in urine. Cancer Res., *53:* 5297–5299, 1993.
35. Lee, J. S., Yoon, A., Kalapurakal, S. K., Ro, J. Y., Lee, J. J., Tu, N., Hittelman, W. N., and Hong, W. K. Expression of p53 oncoprotein in non-small-cell lung cancer: a favorable prognostic factor. J. Clin. Oncol., *13:* 1893–1903, 1995.
36. Nishio, M., Koshikawa, T., Kuroishi, T., Suyama, M., Uchida, K., Takagi, Y., Washimi, O., Sugiura, T., Ariyoshi, Y., Takahashi, T., Ueda, R., and Takahashi, T. Prognostic significance of abnormal p53 accumulation in primary, resected non-small-cell lung cancers. J. Clin. Oncol., *14:* 497–502, 1996.
37. Pastorino, U., Andreola, S., Tagliabue, E., Pezzella, F., Incarbone, M., Sozzi, G., Buyse, M., Menard, S., Pierotti, M., and Rilke, F. Immunocytochemical markers in stage I lung cancer: relevance to prognosis. J. Clin. Oncol., *15:* 2858–2865, 1997.
38. Adachi, M., Taki, T., Huang, C., Higashiyama, M., Doi, O., Tsuji, T., and Miyake, M. Reduced integrin α3 expression as a factor of poor prognosis of patients with adenocarcinoma of the lung. J. Clin. Oncol., *16:* 1060–1067, 1998.
39. Tanaka, F., Kawano, Y., Li, M., Takata, T., Miyahara, R., Yanagihara, K., Ohtake, Y., Fuuse, T., and Wada, H. Prognostic significance of apoptotic index in completely resected non-small-cell lung cancer. J. Clin. Oncol., *17:* 2728–2736, 1999.
40. Yuan, A., Yu, C. J., Kuo, S. H., Chen, W. J., Lin, F. Y., Luh, K. T., Yang, P. C., and Lee, Y. C. Vascular endothelial growth factor 189 mRNA isoform expression specifically correlates with tumor angiogenesis, patient survival, and postoperative relapse in non-small-cell lung cancer. J. Clin. Oncol., *19:* 432–441, 2001.
41. Schneider, P. M., Praeuer, H. W., Stoeltzing, O., Boehm, J., Manning, J., Metzger, R., Fink, U., Wegerer, S., Hoelscher, A. H., and Roth, J. A. Multiple molecular marker testing (p53, C-Ki-ras, c-erbB-2) improves estimation of prognosis in potentially curative resected non-small cell lung cancer. Br. J. Cancer, *83:* 473–479, 2000.
42. Siegfried, J. M., Weissfeld, L. A., Singh-Kaw, P., Weyant, R. J., Testa, J. R., and Landreneau, R. J. Association of immunoreactive hepatocyte growth factor with poor survival in resectable non-small cell lung cancer. Cancer Res., *57:* 433–439, 1997.
43. Volm, M., Rittgen, W., and Drings, P. Prognostic value of ERBB-1, VEGF, cyclin A, FOS, JUN, and MYC in patients with squamous cell lung carcinomas. Br. J. Cancer, *77:* 663–669, 1998.