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Prognostic Impact of Fibroblast Growth Factor 2 in NSCLC: Co-Expression with VEGFR-3 and PDGF-B Predicts Poor Survival.

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ABSTRACT

Purpose: Fibroblast growth factor 2 (FGF2; basic fibroblast growth factor, b-FGF) and its main receptor FGFR-1 are important in both hemangiogenesis and lymphangiogenesis. Murine studies have indicated a close interplay between both FGF2 and platelet-derived growth factor –B (PDGF-B) as well as FGF2 and vascular endothelial growth factor -3 (VEGFR-3). This study investigates the prognostic impact of FGF2 and FGFR-1 in tumor cells and tumor stroma of resected non-small cell lung carcinomas (NSCLC) and explores the importance of their co-expression with VEGFR-3 or PDGF-B.

Methods: Tumor tissue samples from 335 resected patients with stage I to IIIA NSCLC were obtained and tissue microarrays were constructed from duplicate cores of tumor cells and tumor-related stroma from each specimen. Immunohistochemistry was used to evaluate the expression of the molecular markers FGF2, FGFR-1, VEGFR-3 and PDGF-B.

Results: In univariate analyses, high tumor cell FGF2 expression ($P = 0.015$) was a negative prognostic indicator for disease-specific survival (DSS). In tumor stroma, high FGF2 ($P = 0.024$) expression correlated with good prognosis. In multivariate analyses, high expression of FGF2 in tumor cells ($P = 0.038$) was an independent negative prognostic factor whereas increased FGF2 in stroma ($P = 0.015$) was a positive prognosticator. Tumor cell co-expressions of FGF2/VEGFR-3 ($P < 0.001$) and FGFR-1/PDGF-B ($P = 0.002$) were significant indicators of poor prognosis.

Conclusions: Expression of FGF2 in tumor cells is an independent negative prognostic factor, and the co-expressions of FGF2/VEGFR-3 and FGFR-1/PDGF-B are strongly associated with poor survival in NSCLC patients.

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in both men and women.[1] The most important prognostic variable for survival in non-small cell lung cancer (NSCLC) has been tumor stage, primarily because early stage disease is amenable to complete surgical resection and hitherto only patients who undergo curative surgery have a significant potential for cure.[2,3] Several biochemical and clinical characteristics have been investigated to assess their prognostic and/or predictive relevance. In the era of new targeted therapies, identifying the patients most likely to benefit from such treatment is becoming increasingly important.

Angiogenesis, the process of new blood vessel formation from pre-existing ones, plays a key role in tumor growth.[4] The fibroblast growth factor (FGF) family represents a group of heparin-binding, multifunctional polypeptides with mitogen activity which also is involved in angiogenesis.[5] Fibroblast growth factor 2 (FGF2; basic fibroblast growth factor, b-FGF) is considered a potent stimulator of angiogenesis and binds with high affinity mainly to fibroblast growth factor receptor-1 (FGFR-1), a tyrosine kinase receptor.[4] FGF2 may exert its effect on endothelial cells via a paracrine mode as a consequence to its release by tumor and stromal cells. It is also suggested that FGF2 plays an autocrine role in endothelial cells.[4,6]

Previous data on FGF2's prognostic impact in NSCLC has been conflicting.[7-12] Some studies report tumor cell FGF2 expression to correlate with poor survival [8,10,11] while other studies find no such association.[9,12] In one study, however, an inverse correlation between stromal FGF2 expression and lymph node metastasis was observed.[13] Several NSCLC studies have explored the prognostic role of elevated serum-FGF2, but no consensus has been reached.[7,14-18]

Although the activity of individual angiogenic factors is relatively well studied, less is known about the interplay between various tumor-produced angiogenic factors and their cooperative efforts in promoting tumor neovascularization. Interestingly, a recent study using

murine fibrosarcomas reports a reciprocal interaction between FGF2 and PDGF-B through their tyrosine kinase receptors, FGFR-1 and PDGFR-β.[19,20]

An intimate cross-talk between FGF2 and different members of the VEGFs during hemangiogenesis and lymphangiogenesis has been proposed. Kubo et al.[21] reported that blockade of VEGFR-3 signaling inhibits FGF2-induced lymphangiogenesis in mouse cornea. We have previously reported on the importance of VEGFs and PDGFs and their receptors in both tumor cells and stroma.[22-24] In this study, our aim was, based on appealing preclinical results, to explore the 1) prognostic significance of FGF2 and FGFR-1 expression in both tumor cells and stroma of resected NSCLC patients and 2) their co-expression with PDGF-B and VEGFR-3.

PATIENTS AND METHODS

Patients and Clinical Samples

Primary tumor tissues from anonymized patients diagnosed with NSCLC pathologic stage I to IIIA at the University Hospital of Northern Norway (UNN) and Nordland Central Hospital (NLCH) from 1990 through 2004 were used in this retrospective study. In total, 371 patients were registered from the hospital database. Of these, 36 patients were excluded from the study due to: (i) Radiotherapy or chemotherapy prior to surgery (n = 10); (ii) Other malignancy within five years prior to NSCLC diagnosis (n = 13); (iii) Inadequate paraffin-embedded fixed tissue blocks (n = 13). Adjuvant chemotherapy was not introduced in Norway during this period (1990 – 2004). Thus, 335 patients with complete medical records and adequate paraffin-embedded tissue blocks were eligible.

This report includes follow-up data as of September 30, 2005. The median follow-up was 96 (range 10-179) months. Complete demographic and clinical data were collected retrospectively. Formalin-fixed and paraffin-embedded tumor specimens were obtained from the archives of the Departments of Pathology at UNN and NLCH. The tumors were staged according to the International Union Against Cancer's TNM classification and histologically subtyped and graded according to the World Health Organization guidelines.[25] The National Data Inspection Board and The Regional Committee for Research Ethics approved the study.

Microarray construction

All lung cancer cases were histologically reviewed by two pathologists (S.A.S) and (K.A.S.) and the most representative areas of tumor cells (neoplastic epithelial cells) and tumor stroma were carefully selected and marked on the hematoxylin and eosin (H/E) slide and sampled for the tissue microarray blocks (TMAs). The TMAs were assembled using a tissue-arraying instrument (Beecher Instruments, Silver Springs, MD). The Detailed

methodology has been previously reported.[22] Briefly, we used a 0.6 mm diameter stylet, and the study specimens were routinely sampled with two replicate core samples (different areas) of neoplastic tissue and two of tumor stroma. Both normal lung tissue localized distant from the primary tumor, and one slide with normal lung tissue samples from 20 patients without a cancer diagnosis, were used as negative controls.

To include all core samples, eight tissue array blocks were constructed. Multiple 5-µm sections were cut with a Micron microtome (HM355S) and stained by specific antibodies for immunohistochemistry (IHC) analysis.

Immunohistochemistry (IHC)

The applied antibodies were subjected to in-house validation by the manufacturer for IHC analysis on paraffin-embedded material. The antibodies used in the study were FGF2 (rabbit polyclonal; AB1458; Chemicon; 1:200) and FGFR-1 (rabbit polyclonal; sc-121; Santa Cruz; 1:50).

Sections were deparaffinised with xylene and rehydrated with ethanol. Antigen retrieval was performed by placing the specimen in 0.01M citrate buffer at pH 6.0 and exposed to microwave heating of 10 min at 250W (FGF2) or heated by pressure boiler of two min (FGFR-1). The DAKO EnVision + System-HRP (DAB) kit was used as endogen peroxidase blocking. As negative staining controls, the primary antibodies were replaced with the primary antibody diluent. Primary antibodies were incubated for 30 min (FGF2) or 60 min (FGFR-1) in room temperature. The kit DAKO EnVision + System-HRP (DAB) was used to visualize the antigens. This was followed by application of liquid diaminobenzidine and substrate-chromogen, yielding a brown reaction product at the site of the target antigen. Finally, all slides were counterstained with hematoxylin to visualize the nuclei. For each

antibody, included negative staining controls, all TMA staining were performed in a single experiment.

Scoring of IHC

By light microscopy, representative and viable tissue sections were scored semiquantitatively for cytoplasmic staining. The dominant staining intensity in both tumor cells and stromal cells was scored as: 0 = negative; 1 = weak; 2 = intermediate; 3 = strong (Figure 1). The cell density of the stroma was scored as: 1 = low density; 2 = intermediate density; 3 = high density (Figure 1). All samples were anonymized and independently scored by two pathologists (S. Al-Saad and K. Al-Shibli). In case of disagreement, the slides were re-examined and a consensus was reached by the observers. In most tumor cores as well as in some stromal cores there is a mixture of stromal cells and tumor cells. However, by morphological criteria we have only scored staining intensity of tumor cells in tumor cores and intensity and density of stromal cells in stromal cores. When assessing a variable for a given core, the observers were blinded to the scores of the other variables and to outcome. The interobserver scoring agreement has previously been found valid in the same TMA-blocks for one ligand and one receptor with similar cytoplasmic staining.[22] Mean score for duplicate cores from each individual was calculated separately in tumor cells and stroma. High expression in tumor cells was defined as score >1 (FGFR-1) or = 3 (FGF2). Stromal expression was calculated by summarizing density score (1-3) and intensity score (0-3) prior to categorizing into low and high expression. High stromal expression was defined as score ≥ 4.5 (FGF2) or > 4 (FGFR-1).

Statistical Methods

All statistical analyses were done using the statistical package SPSS (Chicago, IL), version 15. The Chi-square test and Fishers Exact test were used to examine the association between molecular marker expression and various clinicopathological parameters. Univariate analysis was done by using the Kaplan-Meier method, and statistical significance between survival curves was assessed by the log rank test. Disease-specific survival (DSS) was determined from the date of surgery to the time of lung cancer death. To assess the independent value of different pretreatment variables on survival, in the presence of other variables, multivariate analysis was carried out using the Cox proportional hazards model. Only variables of significant value from the univariate analysis were entered into the Cox regression analysis. Probability for stepwise entry and removal was set at .05 and .10, respectively. The significance level was set at $p < .05$.

RESULTS

Clinocopathologic Variables

Demographic, clinical, and histopathologic variables are shown in Table 1. The median age was 67 (range, 28-85) years and the majority of patients were male (75%). The NSCLC tumors comprised 191 squamous cell carcinomas (SCCs), 95 adenocarcinomas, 31 large-cell carcinomas (LCCs) and 18 bronchioalveolar carcinomas (BACs). Due to nodal metastasis or non-radical surgical margins, 59 (18%) patients received postoperative radiotherapy.

Expression of FGF2 and FGFR-1 and their Correlations

FGFR-1 and FGF2 were expressed in the cytoplasm of tumor cells. Based on morphological criteria, FGFR-1 showed primarily moderate staining intensity in pneumocytes in control cores. Lymphocytes showed all degrees of staining intensity, approximately 1/3 was negative, 1/3 was weakly positive and 1/3 moderately to strongly positive. Macrophages and plasma cells were stained strongly positive in both control and stromal cores. Bronchial epithelium showed moderate or positive staining intensity while endothelial blood vessel cells in control cores were mostly negative and tumor cells weakly positive. Fibroblast-like cells were weakly stained in both control cores and tumor tissue.

There was a moderate FGF2 expression in pneumocytes. Lymphocytes were approximately 50% weakly positive and 50% negative in control cores, while near all lymphocytes showed moderate to strong staining in tumor stroma. Plasma cells and macrophages showed moderate to strong staining in both control cores and tumor stroma. Bronchial epithelium showed weak staining intensity while the endothelium was primarily

weakly positive in both control cores and tumor stroma. Fibroblast-like cells were generally weakly stained in both control cores and tumor tissue.

Tumor or stromal cell FGF2 or FGFR1 expression did not correlate with age, performance status, tumor differentiation or vascular infiltration. Tumor cell FGF2 was more frequently expressed in node positive patients (high expression; N0 6 %, N1 15 %, N2 15 %, P = .024). Besides, stromal expression of FGF2 was significant reduced in LCC (high expression; LCC 7 %, BAC 28 %, SCC 29 %, adenocarcinomas 20 %, P < 0.029), T2-tumors (high expression; T1 32%, T2 20%, T3 33%, P = 0.044) and tumors with positive surgical margins (high expression; margins free 26 %, margins not free 7 %, P = 0.016).

Tumor cell FGFR-1 was more frequently expressed in females (high expression; females 82%, males 64 %, P = 0.004), in patients without weight loss (high expression; weight loss 50%, no weight loss 70%) and in BAC and adenocarcinomas (high expression; LCC 58 %, BAC 89 %, SCC 62 %, adenocarcinomas 82 %, P < 0.001). Stromal FGFR-1 was more often expressed in tumors with T1-status (high expression; T1 33%, T2 21%, T3 18 %, P = 0.049).

Univariate Analysis

Among the clinical variables, shown in Table 1, performance status (P = 0.04), differentiation (P = 0.001), surgical procedure (P = 0.0009), pathological stage (P < 0.0001), T-stage (P = 0.002), N-stage (P < 0.0001), vascular infiltration (P = 0.0005) and postoperative radiotherapy (P = 0.002) were all significant prognostic indicators for DSS. The influence on survival by tumor cell and stromal expression of FGF2 and FGFR-1 are shown in Table 2A and Figure 2. In univariate analyses, tumor cell FGF2 expression (P = 0.015; Fig. 2A) and stromal FGF2 expression (P = 0.024; Fig. 2B) were prognostic, but opposite indicators for DSS.

Multivariate Cox Proportional Hazards Analysis

Results of the multivariate analysis are presented in Table 3. Including significant clinicopathological and angiogenic variables from the univariate analysis, tumor cell FGF2 (P = 0.038), stromal FGF2 expression (P = 0.015), performance status (P = 0.012), pathological T-stage (P = 0.02), N-stage (P < 0.001), histological differentiation (P = 0.042) and vascular infiltration (P = 0.005) appeared as independent prognostic factors.

Co-expression of FGF2/ FGFR1 with VEGFR-3 and PDGF-B

Table 2B shows the DSS rates of the patients stratified into four groups according to the basis of high or low FGF2 or FGFR1 expression versus a high or low VEGFR-3 or PDGF-B expression. The co-expression of tumor cell FGF2/VEGFR-3 (P < 0.001), FGF2/PDGF-B (P = 0.002), FGFR-1/VEGFR-3 (P = 0.001), and FGFR-1/PDGF-B (P = 0.002), were all significant prognostic indicators for DSS.

Examining the same combinations of stromal co-expressions, there were no significant associations with survival (stromal FGF2/VEGFR-3, P = 0.10; stromal FGF2/PDGF-B, P = 0.052; stromal FGFR-1/VEGFR-3, P = 0.73; stromal FGFR-1/PDGF-B, P = 0.24).

The co-expression of tumor cell FGF2/VEGFR-3 (high FGF2/high VEGFR-3: N0 2,2%; N1 9,3%; N2 14,8%, P < 0.001) and FGFR-1/VEGFR-3 (high FGFR-1/high VEGFR-3: N0 25%; N1 30%; N2 70 %, P = 0.001) correlated significantly with lymph node metastasis, whereas the co-expression of tumor cell FGF2/PDGF-B (P = 0.07) and FGFR-1/PDGF-B (P = 0.09) tended to, but did not reach a significant level.

DISCUSSION

We present a large-scale study in an unselected population of surgically resected NSCLC patients using high-throughput TMA methodology to examine the prognostic impact of FGF2 and FGFR-1 and their co-expressions with VEGFR-3 and PDGF-B in both tumor cells and stroma. High tumor cell FGF2 expression is an independent negative prognostic indicator for DSS, while high stromal FGF2 expression correlates with a good prognosis. Interestingly, tumor cell co-expressions of both FGF2/VEGFR-3 and of FGFR-1/PDGF-B correlated significantly with a poor prognosis.

The prognostic impact of FGF2 in NSCLC is still controversial. Corroborating our results, some previous studies have found tumor cell FGF2 expression to be a negative prognostic factor.[8,10,11] In a cohort of 119 resected NSCLC patients, Shou et al.[10], reported FGF2 as a negative prognosticator, though only in the univariate analysis. However, in a study of 167 stage I-IV NSCLC adenocarcinomas [11], FGF2 appeared as an independent indicator of poor prognosis while FGFR-1 had a negative prognostic impact in the univariate analysis. Using frozen tissue and ELISA technique, in a cohort of 71 resected NSCLC patients, Iwasaki et al.[8] observed that FGF2 had an independent negative impact on survival. In contrast, other studies revealed no correlation between tumor cell FGF2 expression and survival.[9,12] In a relatively large study, involving 206 resected NSCLC tumors, Volm et al.[12] found FGFR-1, but not FGF2, in univariate analysis to correlate with a poor prognosis. In addition, Kojima et al.[9] did not observe a negative prognostic impact of tumor FGF2 expression in a cohort of 132 stage I NSCLC patients.

Studies on FGF2 serum levels in NSCLC patients have been contradictory with respect to prognostic relevance.[14-18] One study reported high serum level of FGF2 to indicate a favorable prognosis.[14] The latter may be explained by our finding of high stromal FGF2 expression as a favorable prognostic indicator. It can be argued that both stromal and tumor

cell FGF2 may contribute to the serum level of FGF2. To our knowledge, this is the first study reporting stromal FGF2 expression to correlate with a good prognosis in NSCLC. Nevertheless, in 84 stage I-IIIA NSCLC patients, Guddo et al.[13] reported stromal FGF2 to inversely correlate with lymph node metastasis, indicating an inhibitory role in NSCLC progression. Corroborating the findings by Guddo et al.[13], we have previously reported stromal VEGFs and VEGFRs to be correlated with increased survival [22], though the mechanisms behind these findings is not fully understood.

It has to be noted that the stromal expression of each marker is the total expression of the different stromal components, including lymphocytes, macrophages, granulocytes and fibroblast-like cells. Thus, the stromal FGF2 expression may be linked to one or more stromal cell types. Almost all lymphocytes were moderately to strongly FGF2 positive in tumor stroma and activation of the adaptive immune system may suppress malignant cell proliferation.[26] Hence, high stromal FGF2 may to some extent reflect activation of the adaptive immune system, which corroborate our previous results in this cohort.[27]

This is the first study investigating the prognostic impact of the co-expression of FGF2 and VEGFR-3 in a large cohort of cancer patients. Beyond being expressed in the lymphatics, VEGFR-3 is also up-regulated in blood vessels in several cancers.[28,29] FGF2 is well established as an important mediator in angiogenesis, but also considered of importance in lymphangiogenesis. Actually, FGF2 pellets implanted in mouse cornea demonstrated the lymphatics to be more responsive to FGF2 than the blood vessels.[30] It has been demonstrated that cross-talk between VEGFs and FGFs may occur in both hemangiogenesis and lymphanigiogenesis.[4] In a study by Chang et al.[31] it was proposed that the lymphatic activity of FGF2 is mediated by endogenous VEGF-C and VEGF-D, leading to VEGFR-3 activation. Hence, in the study by Kubo et al.[21] administration of anti-VEGFR-3 antibodies inhibited the FGF2 lymphangiogenesis in mouse cornea.

Tumor lymphangiogenesis has been associated with lymphatic metastasis, although the precise mechanism is not fully understood.[21,32] Albeit only 16 patients were in the subgroup of high FGF2 / high VEGFR-3 expression, this co-expression of FGF2 and VEGFR-3 appears strongly associated with poor survival. In our previous reports, tumor cell VEGFR-3 expression correlated with both nodal status and survival.[22,23] Herein, we find FGF2 alone and the co-expression of FGF2/VEGFR-3 and FGFR-1/VEGFR-3 to be significantly associated with lymph node metastasis. Actually, in the group of N2 patients, 70 % (19 /27) of the patients had high FGFR-1/high VEGFR-3 expression, indicating lymphangiogenesis as a plausible contributor to poor survival.

Of interest, Nissen et al.[20] recently reported a reciprocal interaction between FGF2 and PDGF-B in a murine tumor model, leading to neovascularization and metastasis. The simultaneous overexpression of FGF2 and PDGF-B resulted in a formation of high-density primitive vascular plexuses, which were poorly coated with pericytes and vascular smooth muscle cells (VSMCs). The underlying mechanisms of this reciprocal interaction involve FGF2 associated upregulation of PDGF receptors in endothelial cells and PDGF-B associated upregulation of FGFR-1 in VSMCs. In our study, there is co-expression of high FGF2 / high PDGF-B in only eight patients. But these patients had a significantly shortened survival (37% 5-year survival) when compared to the low FGF2 / low PDGF-B group (62% 5-year survival, $P = 0.002$). The fact that 86% (24/28) of patients with high tumor cell FGF2 expression also had high tumor cell FGFR-1 expression indicates an autocrine loop in the tumor cells. Although these findings are related to tumor cell expression, we may speculate that high production of these angiogenic factors in the tumor cells may also act in a paracrine fashion to stimulate pericytes, VSMCs and endothelial cells.

The VEGFs, PDGFs and FGFs are all essential in tumor development and different novel targeted therapies aim to inhibit one ore more of these angiogenic markers. Herein,

tumor cell FGF2 expression emerged as an independent negative prognostic factor for stage I-IIIA NSCLC while high stromal FGF2 expression favors a good prognosis. Supporting previous preclinical findings, we have for the first time shown that co-expressions of FGF2/VEGFR-3 and FGFR-1/PDGF-B appear to be significant prognosticators in NSCLC. Based on these results, a multitargeted antiangiogenic approach may be more promising than inhibiting single targets in the treatment of NSCLC patients.

TABLES

Table 1. Prognostic clinicopathologic variables as predictors for disease-specific survival in 335 NSCLC patients (univariate analysis; log-rank test).

Characteristic	Patients (n)	Patients (%)	Median survival (months)	5-Year survival (%)	P
Age					
≤ 65 years	156	47	104	57	0.62
> 65 years	179	53	NR	58	
Sex					
Female	82	25	127	65	0.19
Male	253	75	84	55	
Smoking					
Never	15	5	19	43	0.13
Current	215	64	NR	60	
Former	105	31	84	54	
Performance status					
ECOG 0	197	59	NR	62	0.04
ECOG 1	120	36	61	52	
ECOG 2	18	5	36	40	
Weight loss					
< 10%	303	90	127	57	0.92
> 10%	32	10	NR	57	
Histology					
SCC	191	57	NR	65	0.30
Adenocarcinoma	95	28	52	44	
BAC	18	5	NR	67	
LCC	31	9	84	54	
Differentiation					
Poor	138	41	48	48	0.001
Moderate	144	43	NR	64	
Well	53	16	NR	65	
Surgical procedure					
Lobectomy + Wedge*	243	73	NR	61	0.0009
Pneumonectomy	92	27	35	46	
Stage					
I	212	63	NR	68	< 0.0001
II	91	27	41	46	
IIIa	32	10	18	22	
Tumor status					
1	90	27	NR	75	0.002
2	218	65	84	52	
3	27	8	42	43	
Nodal status					
0	232	69	NR	66	< 0.0001
1	76	23	37	43	
2	27	8	18	20	
Surgical margins					
Free	307	92	127	58	0.34
Not free	28	8	64	51	
Vascular infiltration					
No	284	85	NR	61	0.0005
Yes	51	15	25	35	
Postoperative radiotherapy					
No	276	82	NR	61	0.002
Yes	59	18	41	42	

NR, not reached
* Wedge, n = 10
Abbreviations: SCC; squamous cell carcinoma; BAC, bronchioalveolar carcinoma; LCC, large-cell carcinoma

Table 2. Tumor cell and stromal angiogenic markers as predictors for disease-specific survival in 335 NSCLC patients (univariate analysis; log-rank test).

Marker expression	Patients (n)	Patients (%)	Median survival (months)	5-Year survival (%)	P
A					
FGF2					
Tumor					0.015
Low	307	92	NR	59	
High	28	8	24	37	
Stroma					0.024
Low	253	76	83	53	
High	82	24	NR	70	
FGFR-1					
Tumor					0.15
Low	105	31	NR	61	
High	230	69	83	56	
Stroma					0.37
Low	254	76	104	56	
High	80	24	127	63	
Missing	1	0			
B					
FGF2 and VEGFR-3					
Tumor cell					< 0.001
Low FGF2 low VEGFR-3	199	59	NR	64	
Low FGF2 high VEGFR-3	104	31	63	50	
High FGF2 low VEGFR-3	12	4	NR	72	
High FGF2 high VEGFR-3	16	5	22	10	
Missing	4	1			
FGFR-1 and VEGFR-3					
Tumor cell					0.001
Low FGFR-1 low VEGFR-3	85	25	NR	70	
Low FGFR-1 high VEGFR-3	20	6	26	30	
High FGFR-1 low VEGFR-3	126	38	NR	62	
High FGFR-1 high VEGFR-3	100	30	51	48	
Missing	4	1			
FGF2 and PDGF-B					
Tumor cell					0.002
Low FGF2 low PDGF-B	262	78	NR	62	
Low FGF2 high PDGF-B	44	13	32	45	
High FGF2 low PDGF-B	20	6	43	35	
High FGF2 high PDGF-B	8	3	11	37	
Missing	1	0			
FGFR-1 and PDGF-B					
Tumor cell					0.002
Low FGFR-1 low PDGF-B	97	29	NR	61	
Low FGFR-1 high PDGF-B	8	3	NR	63	
High FGFR-1 low PDGF-B	185	55	127	59	
High FGFR-1 high PDGF-B	44	13	21	41	
Missing	1	0			

NR, not reached

Table 3. Results of Cox regression analysis summarizing significant independent prognostic factors.

Factor	Hazard Ratio	95% CI	P
Tumor status			0.02*
1	1.00		
2	1.67	1.02 – 2.74	0.043
3	2.61	1.31 – 5.21	0.006
Nodal status			< 0.001*
0	1.00		
1	2.14	1.41 – 3.27	< 0.001
2	2.75	1.59 – 4.77	< 0.001
Performance status			0.012*
ECOG 0	1.00		
ECOG 1	1.76	1.19 – 2.60	0.005
ECOG 2	1.81	0.81 – 4.02	0.15
Vascular infiltration			
No	1.00		
Yes	2.00	1.23 – 3.22	0.005
Differentiation			0.042*
Poor	1.00		
Moderate	0.60	0.40 – 0.90	0.012
Well	0.73	0.41 – 1.34	0.32
FGF2 Tumor			
Low	1.00		
High	1.80	1.03 – 3.14	0.038
FGF2 Stroma			
Low	1.78	1.12 – 2.83	0.015
High	1.00		

* Overall significance as a prognostic factor

LEGENDS OF FIGURES

Fig. 1: Immunohistochemical analysis of TMA of NSCLC representing different score for tumor cell basic fibroblast growth factor (FGF2) and stromal FGF2; (A) Tumor cell FGF2 score 1; (B) Tumor cell FGF2 score 3; (C) Stromal FGF2 low score (density 1, intensity 0); (D) Stromal FGF2 high score (density 3, intensity 3).

Fig. 2: Disease-specific survival curves according to; (A) tumor cell basic growth factor (FGF2) expression; (B) stromal FGF2 expression.

Fig. 3: Disease-specific survival curves according to; (A) co-expression basic growth factor (FGF2) and vascular endothelial growth factor receptor (VEGFR)-3; (B) co-expression fibroblast growth factor receptor (FGFR)-1 and platelet-derived growth factor (PDGF)-B.

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Figure 1

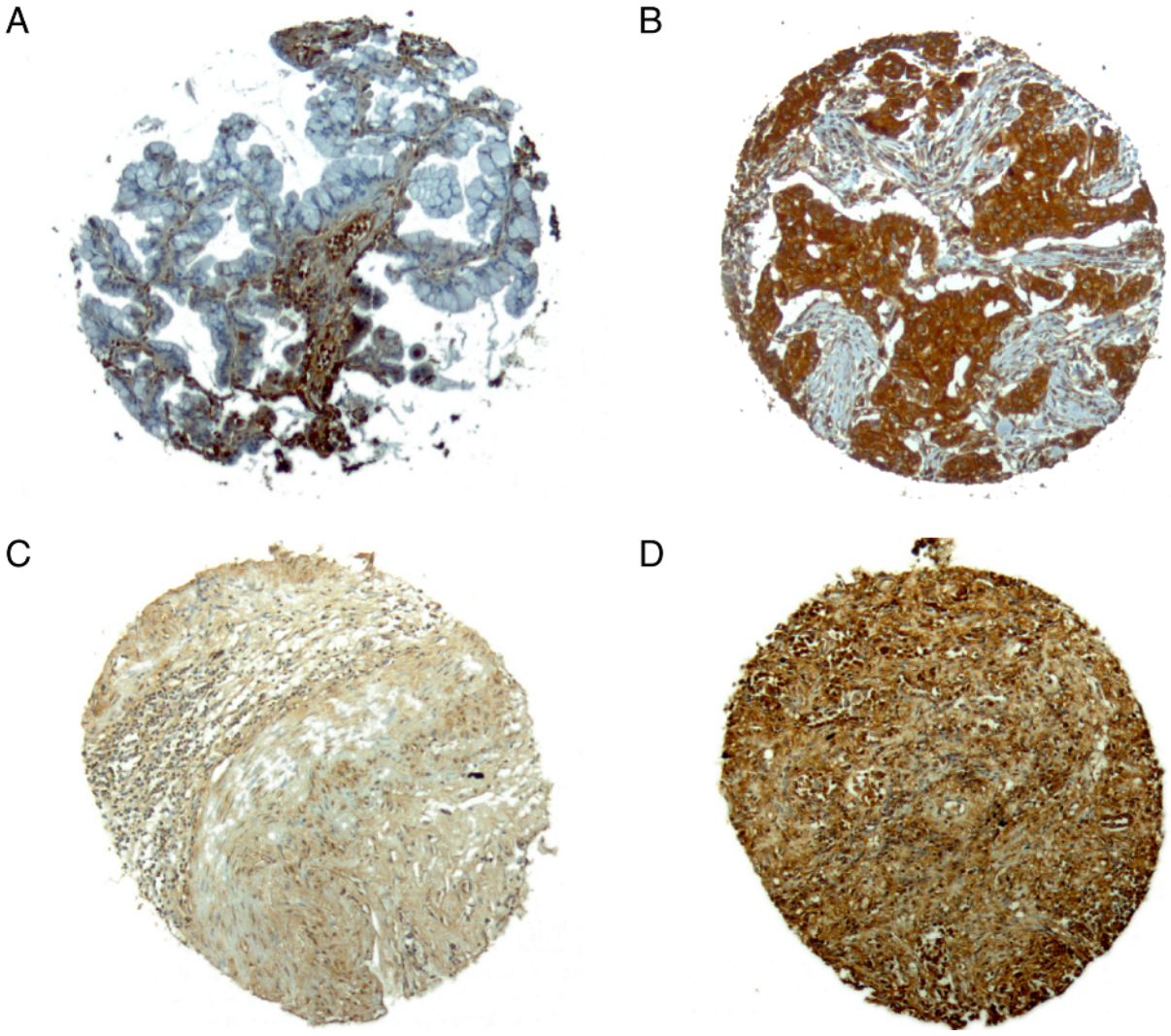


Figure 2

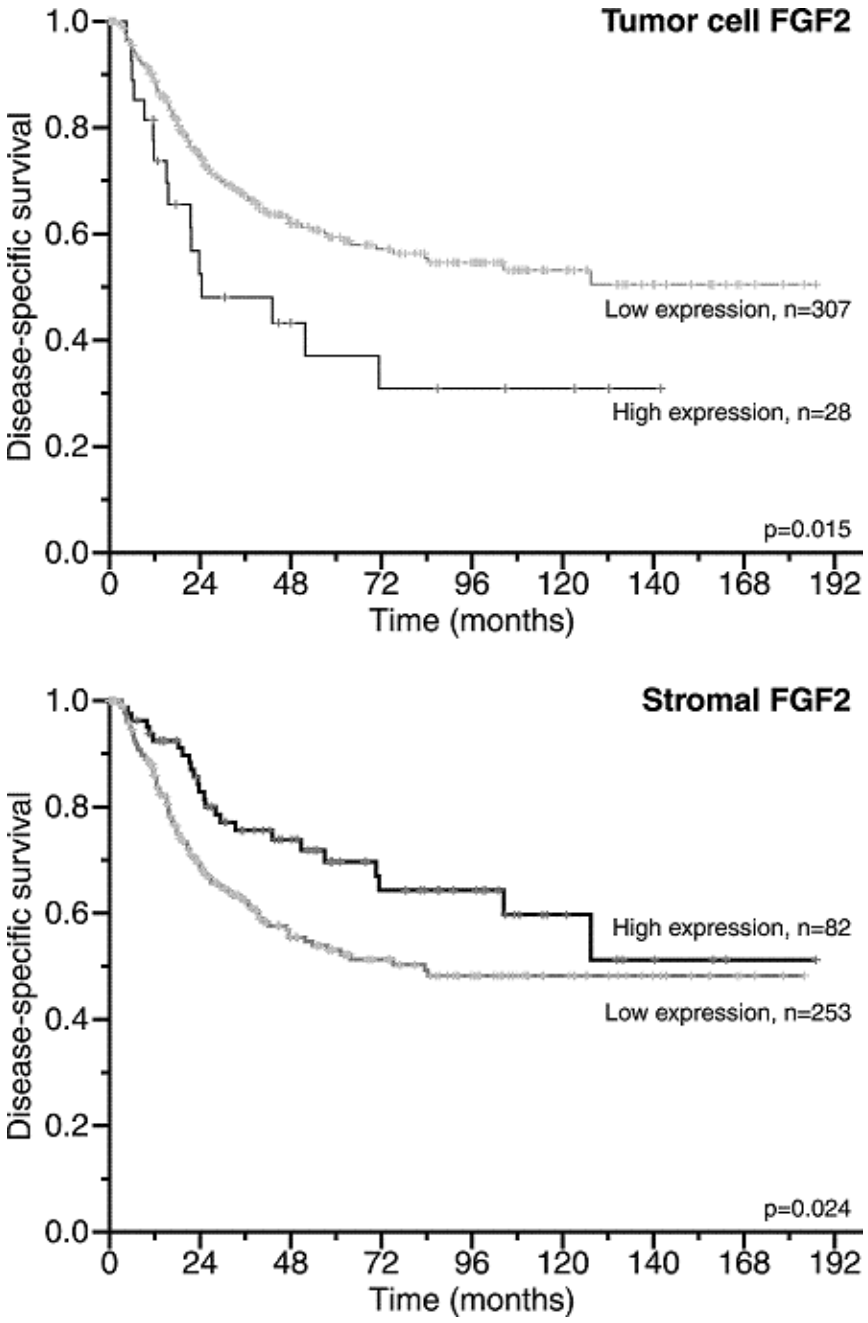
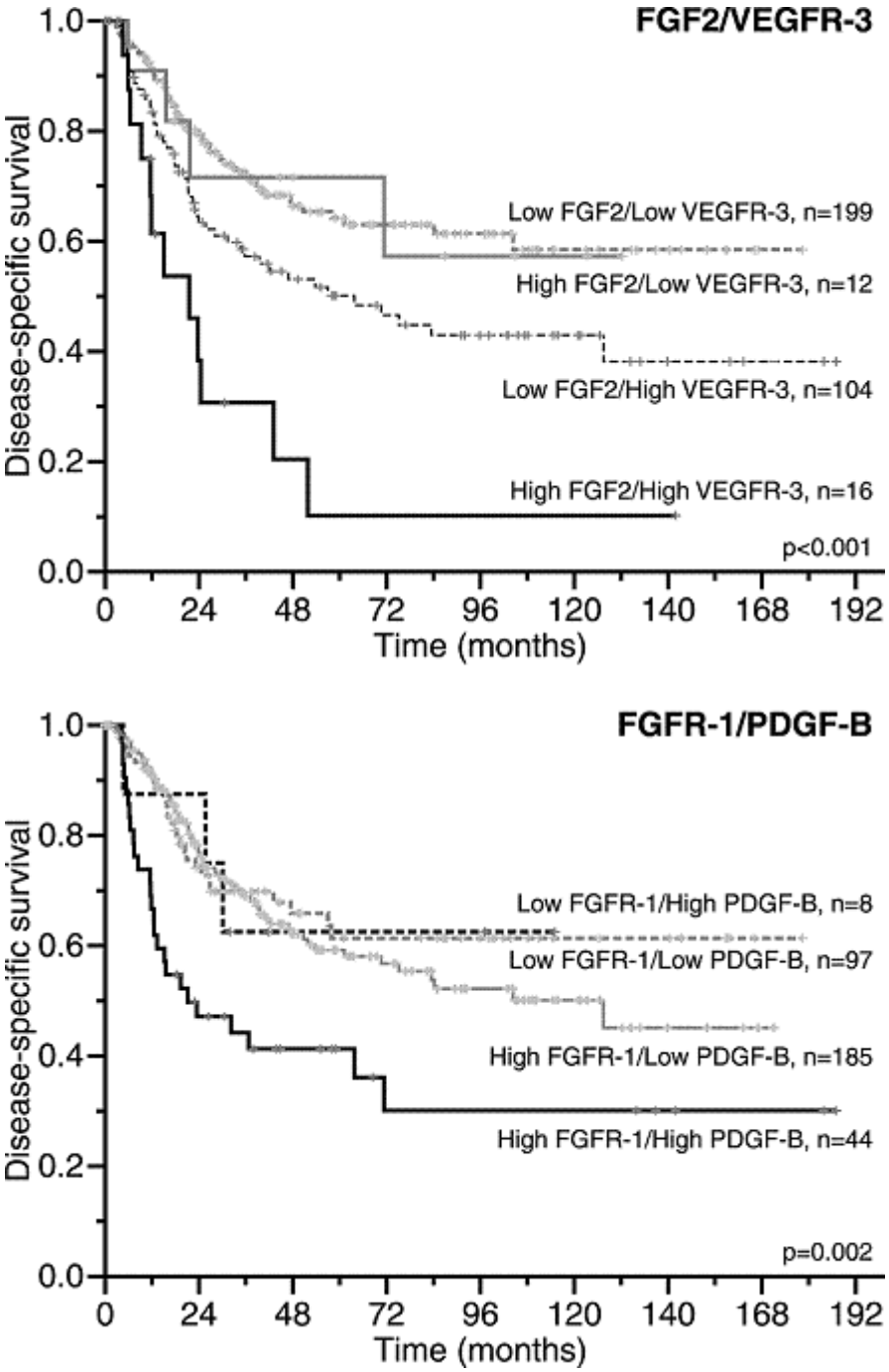

Tumor cell FGF2
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
Low expression, n=307
High expression, n=28
p=0.015
0
24
48
72
96
120
140
168
192
Time (months)
Stromal FGF2
Disease-specific survival
High expression, n=82
Low expression, n=253
p=0.024
Time (months)

Figure 3


FGF2/VEGFR-3
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
Low FGF2/Low VEGFR-3, n=199
High FGF2/Low VEGFR-3, n=12
Low FGF2/High VEGFR-3, n=104
High FGF2/High VEGFR-3, n=16
p<0.001
0
24
48
72
96
120
140
168
192
Time (months)
FGFR-1/PDGF-B
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
Low FGFR-1/High PDGF-B, n=8
Low FGFR-1/Low PDGF-B, n=97
High FGFR-1/Low PDGF-B, n=185
High FGFR-1/High PDGF-B, n=44
p=0.002
0
24
48
72
96
120
140
168
192
Time (months)