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Original Paper

The Basic Fibroblast Growth Factor and its Receptor in Pulmonary Adenocarcinomas: an Investigation of their Expression as Prognostic Markers

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The expression of basic fibroblast growth factor (bFGF) and its receptor, the high-affinity type I basic fibroblast growth factor receptor (FGFR-1), were immunohistologically studied in tissues specimens from 167 patients with a pulmonary adenocarcinoma. Of the 167 specimens, 82 (49%) expressed bFGF and 104 (62%) expressed FGFR-1. bFGF and FGFR-1 were simultaneously expressed in 72 (43%). It was also found that many patients who showed intensely positive staining for bFGF were also positive for FGFR-1, and that the expression of bFGF or FGFR-1 or both was associated with p-stage, T and N factors. The overall prognosis was significantly poorer in the bFGF-positive or FGFR-1-positive patients than in negative patients ($P < 0.01$). The prognosis was also significantly poorer in all patients positive for both bFGF and FGFR-1 than in those negative for both ($P < 0.01$); this was also true for stage I patients ($P < 0.05$). Multivariate analysis showed that bFGF had a significant affect on prognosis, whereas FGFR-1 did not. As FGFR-1 is significantly linked with the bFGF expression, it may be that FGFR-1 interferes with the bFGF effect on survival. These findings suggest that bFGF and FGFR-1 play important roles in tumour progression, and that bFGF expression may be a useful prognostic marker for pulmonary adenocarcinomas. Copyright © 1996 Elsevier Science Ltd

Key words: basic fibroblast growth factor (bFGF), type I basic fibroblast growth factor receptor (FGFR-1), pulmonary adenocarcinoma, proliferation, prognostic marker, immunohistochemical staining
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INTRODUCTION

THE FIBROBLAST growth factor (FGF) family, which consists of polypeptide growth factors with an affinity to heparin and glycosaminoglycans [1, 2], are produced not only by normal cells but also by a number of tumour cells, and are involved in a wide range of activities that includes promotion of mitogenesis, angiogenesis, chemotaxis, cellular differentiation and tissue repair [1]. The basic FGF (bFGF) is closely involved in cancer proliferation and has been reported to be related to progression and prognosis of cancer [3]. Among the FGF receptors (FGFRs), there are five FGFR subtypes, FGFR-1–5 [4]. Although acidic FGF (aFGF) and bFGF bind to and activate FGFR-1 [4], FGFR-1 has been found to bind more readily with bFGF than with aFGF with respect to activation [4]. Further, FGFR-1 is markedly expressed in tumours and has been reported to be involved in their progression [5].

The incidence of lung cancer, of all histological types, is increasing. In the United States, squamous cell carcinoma is the most common lung cancer seen [6], whereas in other countries, especially Japan, adenocarcinomas are the most frequent [7]. According to the 1993 report of an academic investigation by the Japanese Association for Thoracic Surgery, 55% of the 10828 Japanese patients who underwent surgery for lung cancer had an adenocarcinoma and 32% had a squamous cell carcinoma [8]. The incidence of pulmonary adenocarcinoma continues to increase worldwide [9, 10], yet little progress has been made in the understanding of the behaviour of pulmonary adenocarcinomas, and the results of surgical and/or other therapies remain unsatisfactory. There-

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fore, to develop our knowledge and treatment of pulmonary carcinomas, this study evaluated whether immunohistological expression of bFGF and FGFR-1, either singly or jointly, plays a role in the progression and prognosis of pulmonary adenocarcinoma.

PATIENTS AND METHODS

Tissue specimens from 167 patients who underwent surgery for pulmonary adenocarcinoma at the First Department of Surgery of Teikyo University between 1982 and 1989 were studied. Patients who died within 1 month after the operation and those who underwent an exploratory thoracotomy were excluded. Also excluded were patients with a past history of another cancer. The lesions were staged on both the operative findings and pathological findings in the resected specimens, based on the UICC 1987 TNM classification. Thus, the staging of these pulmonary adenocarcinomas was as follows: stage I in 86 patients, stage II in 13, stage IIIa in 44, stage IIIb in 2 and stage IV in 22. The patients consisted of 92 males and 75 females ranging from 28 to 81 years of age (mean 61 years).

The degree of histological differentiation was evaluated according to the World Health Organisation 1982 criteria. However, as the degree of differentiation of an adenocarcinoma sometimes differs among areas of the tumour, the most predominant degree of differentiation in each tumour was the deciding factor. On this basis, the lesions were found to be well differentiated in 84 patients, moderately differentiated in 56, and poorly differentiated in 27. Patients in whom radical surgery had been pre-operatively planned to be a lobectomy or a pneumonectomy with a hilar or a mediastinal lymph node dissection were considered to have manifested operative indications. All patients were followed up for 5–12 years postoperatively and their outcomes were known.

Immunohistological staining

Each resected tissue specimen was fixed with formalin, embedded in paraffin and 3-μm serial sections were cut. The sections were stained with haematoxylin–eosin (H–E) and immunohistological stained for bFGF and FGFR-1, using the mouse anti-bovine bFGF monoclonal antibody (Upstate Biotechnology Inc., Lake Placid, New York, U.S.A.) as the anti-bFGF antibody and the mouse anti-bovine FGFR-1 monoclonal antibody (Chemicon International Inc., Temula, California, U.S.A.) as the anti-FGFR-1 antibody. It should be noted that the bFGF antibody has been confirmed to have a specificity to bovine and human bFGF but not to aFGF [11]. Further, FGFR-1 antibody has been reported to be slightly cross-reactive to the *BEK* gene but is primarily the *FLG* gene product [12,13].

Immunohistological staining for the bFGF and FGFR-1 was based on the ABC method [14] and was performed using a Vestatin Kit (Vector Co. Ltd, Burmingame, California, U.S.A.). Briefly, the sections were deparaffinised, and after inhibition of the endogenous peroxidase, were washed in phosphate-buffered saline (PBS). The sections were then treated with 10% normal rabbit serum (Vector Co. Ltd) and incubated with a 1/50 mouse anti-bovine bFGF monoclonal antibody or mouse anti-FGFR-1 monoclonal antibody as the primary antibody at 4°C overnight. The second reaction was accomplished at room temperature by using biotinated rabbit anti-mouse serum (Vector Co. Ltd). The avidin–biotin peroxidase was placed on to the sections, after which the sections were not disturbed for 60 min, and the sections were then incubated with diaminobenzidine. Methyl green was used to counterstain. Negative control sections were treated with non-immunised mouse IgG as the primary antibody.

Analysis

Immunohistological bFGF and FGFR-1 staining was evaluated under light microscopy by two independent trained observers who were unaware of the stage of the disease or the patient's past history. The degree of immunostaining of the cytoplasms and/or cancer cell nuclei was classified into four grades: −, no cancer cells were stained; +, less than 25% of the cancer cells showed mild staining; ++, from 25 to approximately 74% of the cancer cells showed moderate staining; and +++, 75% or more of the cancer cells showed marked staining.

Associations between the bFGF and FGFR-1 stainings and the bFGF and FGFR-1 associations with the T-, N- and M-factors, and the stage and degree of histological differentiation, were analysed by chi-square test for trend. The survival rate was calculated by Kaplan–Meier's method and compared with the findings of the generalised Wilcoxon test. Variables related to survival were analysed by Cox's proportional hazards regression model with SAS/STAT software (Statistical Analysis System Institute, Cary, North Carolina, U.S.A.) on an NEC PC9801 RA computer. Differences were considered to be significant when the P value was less than 0.05.

RESULTS

Immunohistological staining for bFGF and FGFR-1 was slightly positive in the fibroblasts, the normal pulmonary alveoli, the bronchial epithelium and the vascular endothelium. In the cancer cells of many patients, the cytoplasm showed intense bFGF and FGFR-1 staining (Figure 1). Furthermore, in a few cancer patients, whose cancer cell cytoplasms showed marked staining, bFGF and/or FGFR-1 staining of the nuclei was noted. However, tumour tissue, in which the cytoplasm of the cancer cells were not stained, had no nuclear staining. Cancerous stroma, in a small number of patients, stained for bFGF and/or FGFR-1, and fibroblasts in fibrous stroma were stained for bFGF and/or FGFR-1.

Of the 167 patients, bFGF and FGFR-1 were separately expressed in 82 patients (49%) and 104 patients (62%), respectively, and simultaneously expressed in 72 patients (43%). Positive FGFR-1 staining occurred in 95% of patients whose bFGF staining was graded ++ or +++, and, as shown in Table 1, a significant association was seen between bFGF immunoreactivity grade and the frequency of patients manifesting FGFR-1-positive tumour cells.

Table 2 shows the relationship between bFGF and FGFR-1 immunoreactivity and clinicopathological parameters. The p-stage, and the T- and N-factors, were significantly associated with the bFGF and FGFR-1 expression rates ($P < 0.05$ or $P < 0.01$). Further, the p-stage, T-factor and the N-factor were significantly associated with the simultaneous expression of bFGF and FGFR-1 ($P < 0.05$ or $P < 0.01$).

The overall prognosis was compared between patients who were bFGF- or FGFR-1-positive and patients who were negative for these markers (Figures 2 and 3). The overall prognosis was poorer for patients who were bFGF-positive or FGFR-1-positive than for patients who were negative for either marker ($P < 0.01$), and was clearly poorer for patients positive for both the bFGF and FGFR-1 than for those negative for both markers (Figure 4) ($P < 0.01$).

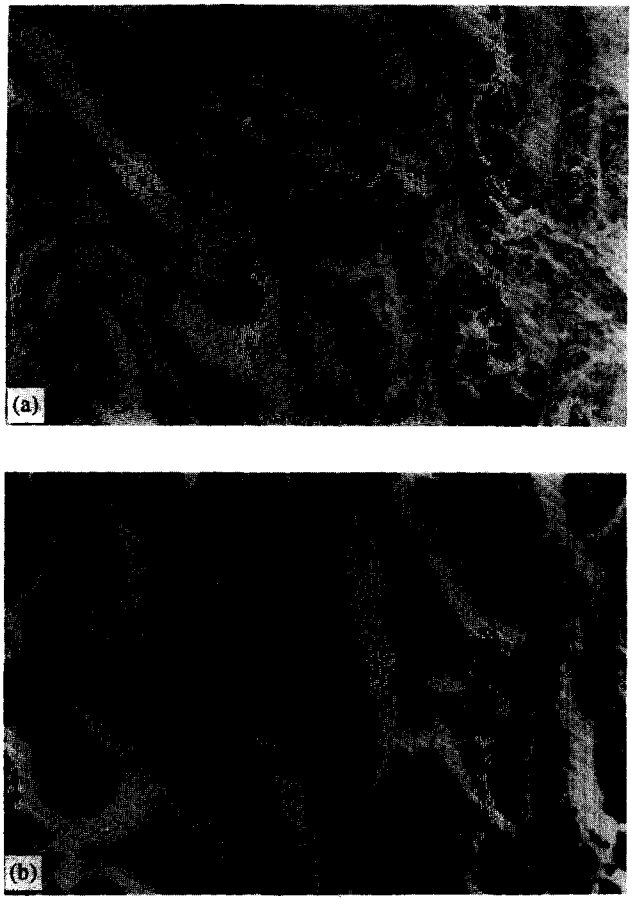

Figure 1. A well-differentiated adenocarcinoma of the lung. Most of the tumour cells simultaneously show intense (a) bFGF and (b) FGFR-1 expression. Magnification ×66.

The p-stage I (T1–T2 N0) prognosis was also compared between patients who were positive and those who were negative for the bFGF or FGFR-1 marker (Figures 5 and 6). p-Stage I bFGF- or FGFR-1-positive patients had a poorer prognosis than p-stage I patients who were negative for either marker ($P < 0.05$). Furthermore, the prognosis was poorer for patients who were positive for both bFGF and FGFR-1 than for those negative for both markers (Figure 7) ($P < 0.05$).

To determine whether bFGF or FGFR-1 expression could serve as an independent prognostic indicator of postoperative survival, a multivariate analysis was performed using the collected data from 143 curatively resected patients. As can be seen in Table 3, the results revealed that the bFGF as well as p-stage were found to be independent prognostic indicators of overall survival (p-stage, $P = 0.0001$; bFGF, $P = 0.0089$).

DISCUSSION

It has been reported that bFGF plays a major role in the infiltration and metastasis of a tumour [15], since bFGF promotes cell proliferation, angiogenesis, fibroblast proliferation, and protease activation and synthesis. Further, in renal cell carcinomas and pancreatic cancers, the value of bFGF expression is said to correlate with the progression of the cancer, and its presence indicates a poor prognosis [3,16]. It is also thought that bFGF produces its effects in cancer cells by binding with FGFR-1, which has a tyrosine kinase activity [4].

The immunohistochemical results of this study have shown that both bFGF and FGFR-1 markedly stained the cytoplasm of the cancer cells, and, in a few cancer patients, the nuclei were also stained. It has also been reported that some of the biological bFGF activities may be instigated by nuclear bFGF-binding proteins or by direct bFGF binding to DNA [17]. In the patients of this study, bFGF was found to be expressed in 49% of the pulmonary adenocarcinomas, and bFGF and FGFR-1 were simultaneously expressed in 43%. FGFR-1 was also detected in many patients who showed an intense bFGF expression, and this study found an association between the bFGF and FGFR-1 expression.

Suzui and associates have reported that bFGF expression and FGFR-1 expression are correlated at the protein as well as at the gene level in pituitary adenoma [18]. In this study, we found that bFGF and FGFR-1 expression were associated with p-stage, T-factor and N-factor, indicating association with tumour size, its stage and lymph node metastasis. In breast cancers, FGFR-1 gene amplification is reported to be associated with lymph node metastasis [19]. Further, in astrocytic tumours, FGFR-1 is reported to be involved in progression [20]. From our results, simultaneous bFGF and FGFR-1 expression was found to be associated with p-stage, T-factor and N-factor. FGFs have been shown to exert an additive and/or synergistic effect on the proliferative actions of the epidermoid growth factor (EGF) [21]. Moreover, FGFR-1 is said to be involved in the autocrine cycle of the EGF receptor (EGFR) [22]. In another study of pulmonary adenocarcinomas, EGFR, EGF and the transforming growth factor σ have been reported to be expressed, and each was shown to be a prognostic factor [23, 24].

Table 1. *Relationship between the bFGF immunoreactivity and the prevalence of FGFR-1 immunoreactivity in pulmonary adenocarcinoma patients*

Grade of bFGF immunoreactivity	Number of cases	Cases with FGFR-1 immunoreactivity	
		Number*	Prevalence %
−	85	32 (10)	37.6 (11.8)
+	41	33 (17)	80.4 (41.5)
++, +++	41	39 (35)	95.1 (85.4)

*Association between the bFGF immunoreactivity and the prevalence of cases with FGFR-1 immunoreactivity (chi-square test for trend, $P < 0.01$).
Numbers in parentheses are numbers of cases showing a ++ or +++ FGFR-1 immunoreactivity grading.

Table 2. Relationship between the bFGF, FGFR-1 immunoreactivities and the clinicopathological factors in the pulmonary adenocarcinoma patients

Variable	Number of cases	Number of cases with immunoreactivity to bFGF	FGFR-1	bFGF + FGFR-1
p-stage†				
I	86	31 (36.0%)	41 (47.7%)	25 (29.0%)
II	13	9 (69.2%)	11 (84.6%)	8 (61.5%)
IIIa	44	27 (61.4%)	33 (75.0%)	25 (56.8%)
IIIb	2	1 (50.0%)	2 (100.0%)	1 (50.0%)
IV	22	14 (63.6%)	17 (77.3%)	13 (59.0%)
P value*		$P < 0.01$	$P < 0.01$	$P < 0.01$
T-factor†				
T1	85	31 (36.5%)	39 (45.9%)	26 (30.6%)
T2	71	43 (60.6%)	56 (78.9%)	38 (53.5%)
T3	11	8 (72.7%)	9 (81.8%)	8 (72.7%)
P value*		$P < 0.01$	$P < 0.01$	$P < 0.01$
N-factor†				
N0	98	40 (40.8%)	52 (53.1%)	34 (34.7%)
N1	13	9 (69.2%)	11 (84.6%)	8 (61.5%)
N2	52	31 (59.6%)	37 (71.2%)	28 (53.8%)
N3	4	2 (50.0%)	4 (100%)	2 (50.0%)
P value*		$P < 0.05$	$P < 0.01$	$P < 0.05$
M-factor†				
M0	145	68 (46.9%)	87 (60.0%)	59 (40.7%)
M1	22	14 (63.6%)	17 (77.3%)	13 (59.1%)
		NS	NS	NS
Differentiation				
Well	84	34 (40.5%)	46 (54.8%)	30 (35.7%)
Moderate	56	33 (58.9%)	41 (73.2%)	28 (50.0%)
Poor	27	15 (55.6%)	17 (63.0%)	14 (51.9%)
P value*		NS	NS	NS

*Chi-square test for trend. †International TNM lung cancer staging classification.
NS, not significant.

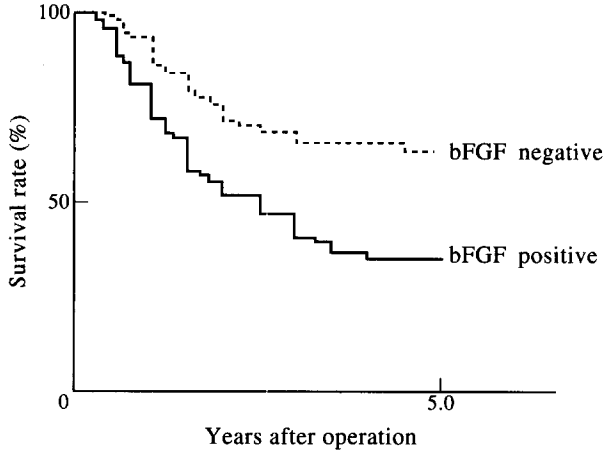

Figure 2. Overall survival curves of the pulmonary adenocarcinoma patients according to bFGF classification. A significant difference was seen between the two groups ($P < 0.01$).

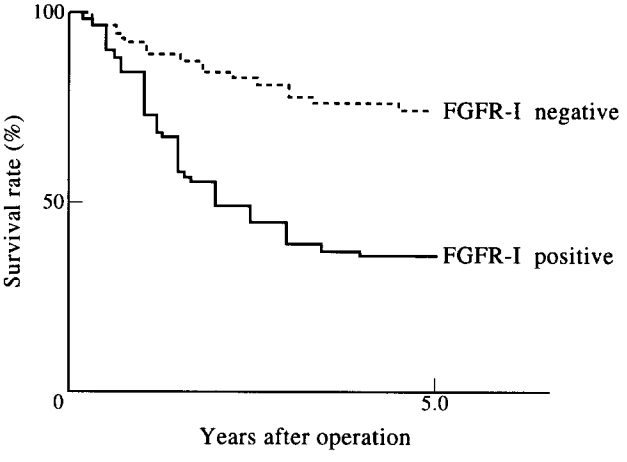

Figure 3. Overall survival curves of the pulmonary adenocarcinoma patients according to FGFR-1 classification. A significant difference was seen between the two groups ($P < 0.01$).

In this study, the overall prognosis of bFGF-positive or FGFR-1-positive patients was poorer than that of bFGF-negative or FGFR-1-negative patients, respectively. Furthermore, the overall prognosis was poorer in bFGF-positive and FGRG-1-positive patients than in bFGF-negative and FGFR-1-negative patients. This was not only true for all patients but for stage I patients. The results of our multivariate analysis revealed that the bFGF expression has a significant affect on prognosis. Further, although the FGFR-1 expression appeared to be a prognostic indicator, our analysis revealed that FGFR-1 is not an independent prognostic indicator. As FGFR-1 is dependent on bFGF, it may be that FGFR-1 interferes with the bFGF effect on survival.

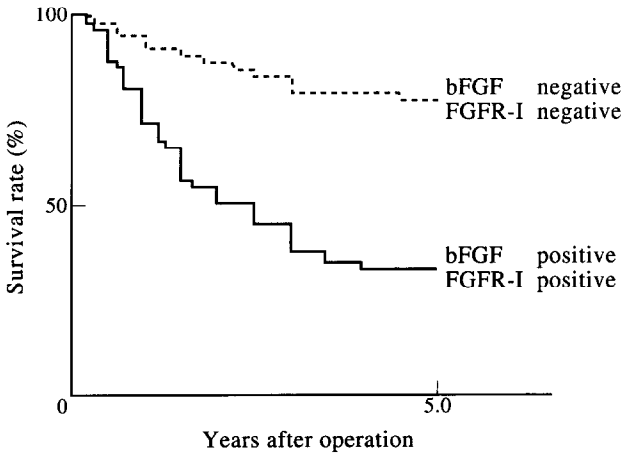

Figure 4. Overall survival curves of the pulmonary adenocarcinoma patients according to bFGF and FGFR-1 classifications. A significant difference was seen between the two groups ($P < 0.01$).

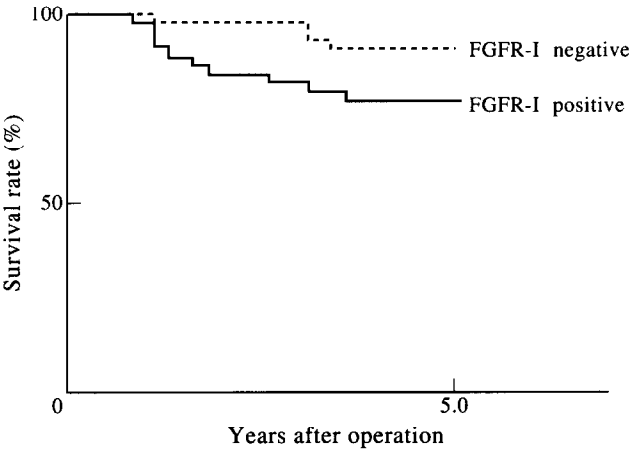

Figure 6. Survival curves of the p-stage I patients according to FGFR-1 classification. A significant difference was noted between the two groups ($P < 0.05$).

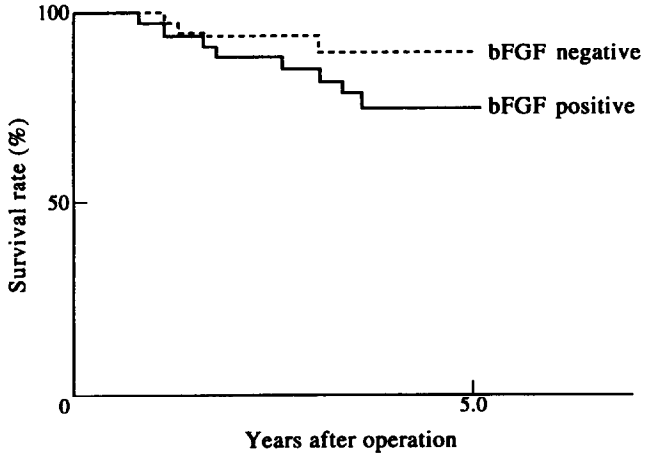

Figure 5. Survival curves of the p-stage I patients according to bFGF classification. A significant difference was noted between the two groups ($P < 0.05$).

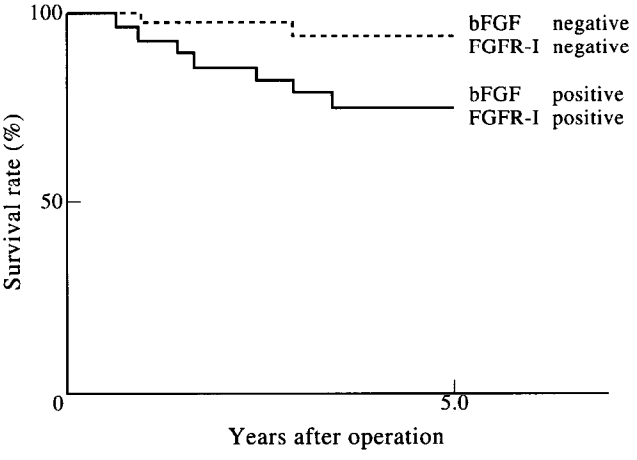

Figure 7. Survival curves of the p-stage I patients according to bFGF and FGFR-1 classifications. A significant difference was seen between the two groups ($P < 0.05$).

It would be useful to know if other lung tumours express bFGF and FGFR-1. bFGF and FGFR-1 mRNA levels should be investigated by *in situ* hybridisation, and the relationship between clinical features and bFGF and FGFR-1 mRNA levels in malignant tumours should be the focus of a future study. As for the findings of this study, it appears that bFGF and FGFR-1 both play an important role in tumour progression and that bFGF expression can be useful as a prognostic marker of pulmonary adenocarcinomas.

Table 3. Results of a multivariate analysis of 143 curatively resected patients using Cox's proportional hazard model*

Variables	Multivariate analysis Parameter estimate	Standard error	χ^2	*P* value
p-stage†	1.000	0.157	40.751	0.0001
bFGF	0.817	0.312	6.837	0.0089
FGFR-1	0.425	0.378	1.265	0.2607

*Patients who died within 1 month postoperatively were excluded. †International TNM lung cancer staging classification.

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