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Biology of Human Tumors

Clinical Cancer Research

FGFR1/3 Tyrosine Kinase Fusions Define a Unique Molecular Subtype of Non–Small Cell Lung Cancer

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Abstract

Purpose: The fibroblast growth factor receptor (FGFR)-3 fusion genes have been recently demonstrated in a subset of non–small cell lung cancer (NSCLC). To aid in identification and treatment of these patients, we examined the frequency, clinicopathologic characteristics, and treatment outcomes of patients who had NSCLC with or without *FGFR* fusions.

Experimental Design: Fourteen known *FGFR* fusion variants, including *FGFR1*, *FGFR2*, and *FGFR3*, were detected by RT-PCR and verified by direct sequencing in 1,328 patients with NSCLC. All patients were also analyzed for mutations in *EGFR*, *KRAS*, *HER2*, *BRAF*, *ALK*, *RET*, and *ROS1*. Clinical characteristics, including age, sex, smoking status, stage, subtypes of lung adenocarcinoma, relapse-free survival, and overall survival, were collected.

Results: Of 1,328 tumors screened, two (0.2%) were *BAG4-FGFR1* fusion and 15 (1.1%) were *FGFR3-TACC3* fusion. Six of 1,016 patients with lung adenocarcinoma were *FGFR3-TACC3* fusions and 11 of 312 lung squamous cell carcinoma harbored *BAG4-FGFR1* or *FGFR3-TACC3* fusions. Compared with the *FGFR* fusion-negative group, patients with *FGFR* fusions were more likely to be smokers (94.1%, 16 of 17 patients, $P < 0.001$), significantly associated with larger tumor (>3 cm; 88.2%, 15 of 17 patients, $P < 0.001$) and with a tendency to be more poorly differentiated (53.9%, nine of 17 patients, $P = 0.095$).

Conclusions: *FGFR* fusions define a molecular subset of NSCLC with distinct clinical characteristics. *FGFR* is a druggable target and patients with *FGFR* fusions may benefit from *FGFR*-targeted therapy, which needs further clinical investigation. *Clin Cancer Res; 20(15); 4107–14. ©2014 AACR.*

Introduction

Lung cancer is the leading cause of cancer mortality worldwide (1, 2). Histologically, non–small cell lung cancer (NSCLC), including adenocarcinoma, squamous cell carcinoma (SCC), and large cell carcinoma, is the most common type, accounting for 85% to 90% of the total cases (3). Identification of oncogenes that drive lung cancer and applying to targeted therapies could lead to improvements in outcomes of patients. Discovery of the association between *EGFR* tyrosine kinase inhibitor (EGFR-TKI) therapy and dramatic regression of tumors have enlarged the treatment considerations for patients with lung cancer (4). Moreover, oncogenic fusion genes that involve kinases, including RET targeted by vandetanib and cabozantinib, as well as ALK and ROS1, which are both sensitive to treatment with crizotinib, have also been proved to be effective targets for molecular therapy in lung cancers (5–7). Identification of oncogenic driver genes in NSCLC is becoming increasingly important. Even with broad genotyping, there remains 30% of lung adenocarcinoma from smokers and 88% of lung SCC from East Asian population without any known driver mutations (8, 9).

The basic fibroblast growth factor receptor (FGFR) family is made up of four active members, *FGFR1*, *FGFR2*, *FGFR3*, and *FGFR4*. The activation of these kinases by amplification, mutations, or translocation plays crucial roles in cancer initiation and development (10, 11). Recently, the *FGFR* gene rearrangements, including *FGFR1*, *FGFR2*, and *FGFR3*, were identified in diverse cancers, including glioblastoma, bladder cancer, cholangiocarcinoma, thyroid

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Translational Relevance

Fibroblast growth factor receptor (*FGFR*) tyrosine kinase fusions have been described in a subset of lung tumors. The prevalence, clinicopathologic characteristics, and prognostic implications of *FGFRs* rearranged lung cancers are not well established. *FGFR1/3* fusions occurred in 1.3% of patients with non–small cell lung cancers (NSCLC) and 3.5% of patients with lung squamous cell carcinoma. Lung cancer is the most common cause of cancer-related death worldwide and with an estimated 1,608,800 new cases of lung cancer per year worldwide in 2005; this equates to more than 20,000 new affected individuals per year. Identifying even a small subpopulation of patients with gene fusions who may be potentially responsive to targeted therapy, as exemplified by the discovery of *RET* and *ROS1* fusions, may have major therapeutic relevance.

cancer, oral cancer, breast cancer, head and neck cancer, and lung SCC (12–15). Preclinical studies have demonstrated the role for *FGFR* fusions in tumor initiation and maintenance (12–14). *FGFR* fusions have been shown to sensitize cancer cells to *FGFR* kinase inhibitors PD173074 and pazopanib (16), suggesting that a new subset of cancers may be treatable with *FGFR*-targeted therapy. The Cancer Genome Atlas project demonstrated that six of 222 (2.7%) lung SCCs harbored *FGFR2* or *FGFR3* fusions (15). Most recently, two *FGFR3* fusions were detected in 144 (1.4%) East Asian patients with SCC (17). Because of the small number of *FGFR* fusion-positive cases identified in these studies, the key clinicopathologic characteristics associated with *FGFR* fusions have not yet been established.

Here, we analyzed 1,328 resected NSCLCs for *FGFR1*, *FGFR2*, and *FGFR3* fusions using RT-PCR and described the clinical and pathologic characteristics of patients with NSCLC with *FGFR* fusions. We also compared them with those with mutations in *EGFR*, *KRAS*, *HER2*, *BRAF*, *ALK*, *RET*, and *ROS1*. These studies allowed us to define unique clinicopathologic characteristics associated with *FGFR* fusions.

Patients and Methods

Patients and tissues

From October 2007 to December 2012, a total of 1,328 frozen surgically resected NSCLC tumor tissues, including 1,016 lung adenocarcinomas and 312 SCCs, were prospectively collected in the Department of Thoracic Surgery of Fudan University Shanghai Cancer Center (Shanghai, China). This study was approved by the Institutional Review Board of the Fudan University Shanghai Cancer Center. All patients were provided written informed consent. Three pathologists (Y. Li, L. Shen, and X. Shen) confirmed the diagnosis of adenocarcinoma and SCC by hematoxylin and eosin staining. RNA was extracted from frozen tissues as per standard protocols (RNeasy Mini Kit; Qiagen). Total RNA samples were then reverse transcribed into single-stranded cDNA, using a RevertAid First Strand cDNA Synthesis Kit (Fermentas). cDNAs were used for PCR amplification and sequencing. Clinical and pathologic data were prospectively collected for analyses, including age at diagnosis, sex, smoking history, histologic type, pathologic TNM stage, tumor size, and tumor differentiation. Patients were observed in clinic or by telephone for disease recurrence and survival from the date of diagnosis.

Detection of *FGFR* gene fusions

FGFR1, *FGFR2*, and *FGFR3* gene fusions were detected by RT-PCR. Multiple pairs of primers were designed to cover 14 known *FGFR* fusion variants. To demonstrate *FGFR1* gene fusions, *FGFR1-TACC1*, *FGFR1-FGFR1*, *ERLIN2-FGFR1*, and *BAG4-FGFR1* were explored. 5′ *FGFR2* fusions to *BICC1*, *AFF3*, *CASP7*, *CCDC6*, *KIAA1967*, *OFD1*, and *CIT* were detected and 3′ *FGFR2* fusions to *SLC45A3* were illustrated. *FGFR3* parted with *TACC3* or *BAIAP2L1* was also illustrated.

PCR analysis was performed with KOD plus DNA polymerase (Toyobo). The program to detect transcriptional *FGFR1-TACC1*, *BAG4-FGFR1* was as follows: 94°C for 2 minutes; 98°C for 10 seconds, 60°C for 30 seconds, 68°C for 40 seconds, 40 cycles; and 68°C for 5 minutes. The program to detect transcriptional *FGFR1-FGFR1* fusions was as follows: 94°C for 2 minutes; 98°C for 10 seconds, 60.0°C for 30 seconds, 68°C for 60 seconds, 40 cycles; and 68°C for 5 minutes. The program to detect transcriptional *ERLIN2-FGFR1* was as follows: 94°C for 2 minutes; 98°C for 10 seconds, 60°C for 30 seconds, 68°C for 45 seconds, 40 cycles; and 68°C for 5 minutes. The program to detect transcriptional *FGFR2- BICC1* was as follows: 94°C for 2 minutes; 98°C for 10 seconds, 60°C for 30 seconds, 68°C for 40 seconds, 40 cycles; and 68°C for 5 minutes. The program to detect transcriptional *FGFR2-AFF3*, *FGFR2-CASP7*, *FGFR2-CCDC6*, *FGFR2- KIAA1967*, *FGFR2-OFD1*, *FGFR2-CIT*, and *SLC45A3-FGFR2* was as follows: 94°C for 2 minutes; 98°C for 10 seconds, 60°C for 30 seconds, 68°C for 30 seconds, 40 cycles; and 68°C for 5 minutes. The program to detect transcriptional *FGFR3-TACC3*, *FGFR3-BAIAP2L1* was as follows: 94°C for 2 minutes; 98°C for 10 seconds, 61°C for 30 seconds, 68°C for 30 seconds, 40 cycles; and 68°C for 5 minutes. PCR products were directly sequenced in both forward and reverse directions. All mutations were verified by analysis of an independent PCR isolate. The primers and detailed PCR program are described in the Supplementary Table S1.

Mutational analyses

EGFR (exons 18 to 22), *KRAS* (exons 2 to 3), *HER2* (exons 18 to 21), and *BRAF* (exons 11 to 15) were PCR amplified using cDNA for direct sequencing. All mutated cases were confirmed twice with independent PCR reactions. *ALK*, *RET*, and *ROS1* fusions were analyzed by qRT-PCR plus RT-PCR and confirmed by FISH as we previously reported (18–20).

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IHC analysis

To evaluate IHC analysis for detecting *FGFR* fusions, all *FGFR* fusion-positive samples, as well as 103 additional lung SCCs were subjected to IHC analysis with formalin-fixed paraffin-embedded slides sectioned at 4 μm. The following antibodies were used: FGFR1 (clone: D8E4, 1:500; Cell Signaling Technology), FGFR3 (polyclonal antibody, 1:50; Abcam), FGFR3 (clone: C51F2, 1:50; Cell Signaling Technology), P40 (ΔNP63; Ready-to-use polyclonal antibody, Fuzhou Maxim Biotech, Ltd.), TTF-1 (clone: SPT24, Ready-to-use antibody, Fuzhou Maxim Biotech, Ltd.). All slides were reviewed by two pathologists (Y. Li and L. Shen).

Statistical analysis

Associations between mutations and clinical and pathologic characteristics were analyzed by the χ^2 test or Fisher exact test. Patients diagnosed from April 2008 to December 2011 were included for survival analysis. Survival curves were estimated using the Kaplan–Meier method, with differences in survival assessed by the log-rank test. Data were analyzed using Statistical Package for the Social Sciences Version 16.0 Software (SPSS Inc.) or Prism 5.0 (GraphPad Software Inc). The two-sided significance level was set at $P < 0.05$.

Results

Tumors from 1,328 patients with NSCLC were examined, including 1,016 adenocarcinomas and 312 SCCs. The characteristics for these patients are listed in Supplementary Table S2.

Detection of *FGFR* Fusion in NSCLC

All tumors were examined by RT-PCR and direct sequencing with primer sets covered 14 known *FGFR* fusion variants. Seventeen *FGFR* fusions (2 *BAG4-FGFR1* and 15 *FGFR3-TACC3*) in 1,328 NSCLC were identified. No fusions were found in *FGFR1-TACC1*, *ERLIN2-FGFR1*, *FGFR1-FGFR1*, *FGFR2-AFF3*, *FGFR2-CASP7*, *FGFR2-CCDC6*, *FGFR2-KIAA1967*, *FGFR2-OFD1*, *FGFR2-CIT*, *SLC45A3-FGFR2*, *FGFR2-BICC1*, and *FGFR3-BAIAP2L1*. Of 312 SCCs, 11 (3.5%) harbored *FGFR1/3* fusions, which included two *BAG4-FGFR1* and nine *FGFR3-TACC3* fusions. Six patients (0.6% of 1016) with lung adenocarcinoma were identified to harbor *FGFR3-TACC3* fusions (Fig. 1 and Supplementary Fig. S1). Details of the 17 *FGFR1/3* fusion-positive NSCLCs are listed in Table 1. The histology of 17 tumors with *FGFR1* or *FGFR3* fusions was confirmed by IHC biomarkers (p40 and TTF-1).

Clinicopathologic characteristics of patients with *FGFR* fusion in NSCLCs

Of 1,328 NSCLCs, 838 patients are found to harbor mutations in *EGFR*, *KRAS*, *BRAF*, *HER2*, *FGFR*, *ALK*, *RET*, or *ROS1*. There are 36.9% (490 of 1,328) with unknown oncogenic mutations (Fig. 2). As shown in Table 2 and Supplementary Table S3, patients with *FGFR1/3* fusion-positive tumors displayed unique characteristics. These patients were predominantly SCCs with relatively equal age and stage distribution. *FGFR*-positive cases had a significantly larger primary lesion (>3 cm) than those with *FGFR* fusion negative ($P < 0.001$). *FGFR* fusion genes were significantly more frequent in males (16 of 754, 2.1%) than in females (1 of 574, 0.2%, $P = 0.002$) and in smokers (16 of 580 patients; 2.8%) than in never smokers (1 of 748 patients; 0.15%; $P < 0.0001$). Because smoking and male sex correlated with squamous histology, we undertook a multivariate analysis, including sex, age at diagnosis, smoking history, tumor size, stage, and histology (Supplementary

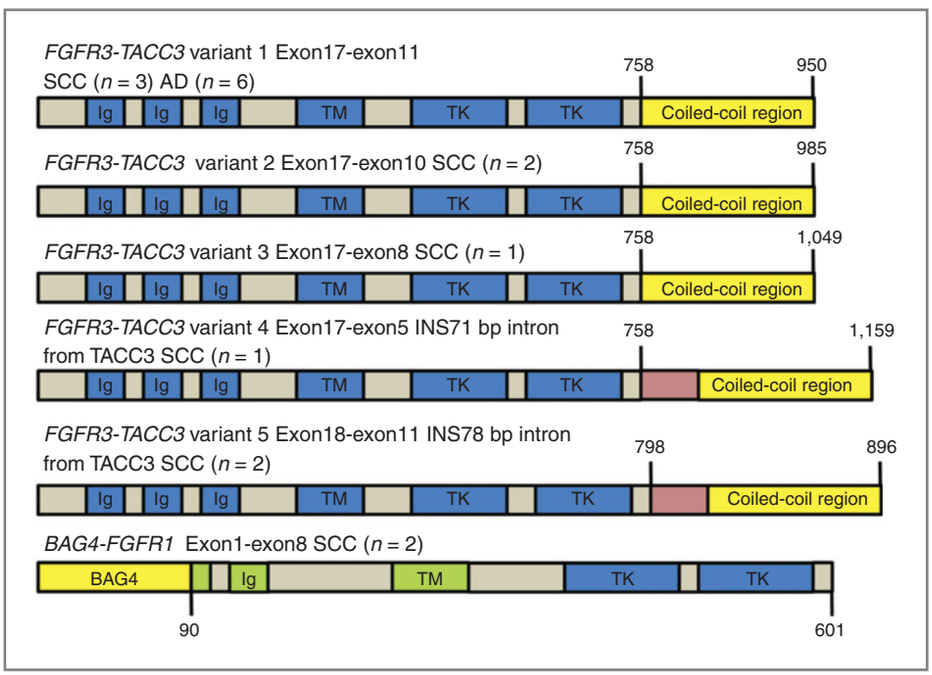

Figure 1. *FGFR3-TACC3* fusions variants in NSCLC.

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Table 1. Clinicopathologic details of 17 patients with NSCLC with *FGFR* fusions

Patient No.	Age, y	Sex	Smoking (pack-years)	Stage	Tumor size (cm)	Lymph node status	Histology	Differentiation	FGFR fusion	IHC score					Subtype
										FGFR1	FGFR3a	FGFR3c	P40	TTF1	
1	48	M	30	II B	8.0	N1	SCC	Well	FGFR3-TACC3*	−	−	−	+	−	
2	70	M	40	I B	3.2	0	SCC	Moderate	FGFR3-TACC3Δ	−	+	+	+	−	
3	49	M	20	III A	4.0	N2	SCC	Moderate	FGFR3-TACC3Δ	−	+	−	+	−	
4	55	M	30	III A	5.0	N2	SCC	Poor	FGFR3-TACC3°	−	+	+	+	−	
5	55	M	45	II A	2.0	N1	SCC	Poor	FGFR3-TACC3♀	−	+	+	+	−	
6	65	M	40	I B	5.0	0	SCC	Poor	FGFR3-TACC3*	+	+	+	+	−	
7	62	M	45	II B	6.0	N1	SCC	Poor	FGFR3-TACC3♂	−	+	+	+	−	
8	62	M	35	II B	8.0	0	SCC	Moderate	FGFR3-TACC3♂	−	+	+	+	−	
9	46	M	40	I A	3.0	0	SCC	Moderate	FGFR3-TACC3Δ	+	−	−	+	−	
10	75	M	50	II A	4.0	N1	AD	Poor	FGFR3-TACC3Δ	−	+	+	−	+	Solid
11	64	M	40	III A	3.5	N2	AD	Poor	FGFR3-TACC3Δ	+	+	−	−	+	Papillar + solid
12	56	M	5	I B	4.0	0	AD	Moderate	FGFR3-TACC3Δ	−	+	+	−	+	Acinar
13	52	M	60	I B	5.0	0	AD	Moderate	FGFR3-TACC3Δ	−	+	−	−	+	Solid
14	62	M	35	II A	6.0	0	AD	Poor	FGFR3-TACC3Δ	−	+	−	−	+	Acinar + solid
15	45	F	0	III A	4.0	N2	AD	Poor	FGFR3-TACC3Δ	−	+	−	−	+	Solid
16	61	M	40	II B	5.5	0	SCC	Moderate	BAG4-FGFR1	−	+	+	+	−	
17	48	M	105	I B	4.0	0	SCC	Poor	BAG4-FGFR1	+	+	−	+	−	

Abbreviation: AD, adenocarcinoma.

*FGFR3-TACC3**, *FGFR3-TACC3* (E18:E11 INS78 bp intron from TACC3).

FGFR3-TACC3Δ, *FGFR3-TACC3* (E17:E11).

FGFR3-TACC3°, *FGFR3-TACC3* (E17:E8).

FGFR3-TACC3♀, *FGFR3-TACC3* (E17:E5 INS71 bp intron from TACC3).

FGFR3-TACC3♂, *FGFR3-TACC3* (E17:E10).

BAG4-FGFR1, *BAG4-FGFR1* (E1:E8).

FGFR3(a): FGFR3 antibody from Abcam.

FGFR3(c): FGFR3 antibody from Cell Signaling Technology.

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Table 2. Clinicopathologic characteristics of patients with NSCLC ($n = 1,328$)

Characteristic	*FGFR* fusion Patients, *n* (%)	*EGFR Mut.* (positive) Patients, *n* (%)	*P*	*KRAS Mut.* (positive) Patients, *n* (%)	*P*	*HER2 Mut.* (positive) Patients, *n* (%)	*P*	*BRAF Mut.* (positive) Patients, *n* (%)	*P*	*FGFR* (negative) Patients, *n* (%)	*P*
Age, y											
≤60	8 (47.1)	296 (47.5)		44 (55.0)		19 (73.1)		4 (33.3)		625 (47.7)	
>60	9 (52.9)	327 (52.5)	0.971	36 (45.0)	0.551	7 (26.9)	0.084	8 (66.7)	0.703	686 (52.3)	0.960
Sex											
Male	16 (94.1)	232 (37.2)		65 (81.3)		3 (11.5)		8 (66.7)		738 (56.3)	
Female	1 (5.9)	391 (62.8)	**<0.001**	15 (18.8)	0.290	23 (88.5)	**<0.001**	4 (33.3)	0.130	573 (43.7)	**0.002**
Smoking status											
Never	1 (5.9)	496 (79.6)		23 (28.8)		25 (96.2)		4 (33.3)		746 (56.9)	
Smoker	16 (94.1)	127 (20.4)	**<0.001**	57 (71.3)	0.357	1 (3.8)	**<0.001**	8 (66.7)	0.198	565 (43.1)	**<0.001**
Histology											
AD	6 (35.3)	609 (97.8)		73 (91.3)		25 (96.2)		12 (100.0)		1010 (77.0)	
SCC	11 (64.7)	14 (2.2)	**<0.001**	7 (8.8)	**<0.001**	1 (3.8)	**<0.001**	0 (0.0)	**<0.001**	301 (23.0)	**<0.001**
Tumor size											
≤3 cm	2 (11.8)	480 (77.0)		50 (62.5)		18 (69.2)		7 (58.3)		830 (63.3)	
>3 cm	15 (88.2)	143 (23.0)	**<0.001**	30 (37.5)	**0.001**	8 (30.8)	**<0.001**	5 (41.7)	0.14	481 (36.7)	**<0.001**
Stage											
I–II	13 (76.5)	434 (69.7)		57 (71.3)		17 (65.4)		8 (66.7)		893 (68.1)	
III–IV	4 (23.5)	189 (30.3)	0.546	23 (28.8)	0.773	9 (34.6)	0.439	4 (33.3)	0.683	418 (31.9)	0.462
Differentiation											
Well-moderate	8 (47.1)	481 (77.2)		49 (61.3)		18 (69.2)		8 (66.7)		870 (66.4)	
Poor	9 (53.9)	142 (22.8)	**0.008**	31 (38.8)	0.280	8 (30.8)	0.146	4 (33.3)	0.296	441 (33.6)	0.095

NOTE: The bold values indicate that the differences are statistically significant.
Abbreviation: AD, adenocarcinoma.

Table S4). Tumor size was an independent predictor of *FGFR1/3* fusions ($P = 0.015$). Smoking also was correlated independently with *FGFR1/3* fusions with borderline significance ($P = 0.093$).

IHC analysis of *FGFR*-fusion–positive NSCLCs

Seventeen *FGFR* fusion-positive samples were subjected to IHC staining for FGFR protein. Of 15 tumors with *FGFR3* fusion, seven were scored as negative to weak and eight as positive using FGFR3 antibody from Cell Signaling Technology (1#). Another FGFR3 antibody from Abcam (2#) was also used which showed that two were negative and thirteen were positive. FGFR1 expression was also examined in tumors with *BAG4-FGFR1*. Of the two *FGFR1*-positive samples, one was positive and the other was negative (Supplementary Fig. S2). Additional 103 lung SCCs without *FGFR* fusions (consecutively collected from July 2011 to December 2012) were also stained for FGFR1 and FGFR3 protein. FGFR1, FGFR3 (1#), and FGFR3 (2#) expressions were detected in 11.7% (12 of 103), 11.7% (12 of 103), and 22.3% (23 of 103), respectively. Expression of FGFR3 was significantly higher in tumors with *FGFR3* fusion than that in those without *FGFR3* fusion with both antibodies ($P < 0.0001$ and 0.023 for *FGFR3* fusion vs. *FGFR3* wild-type). There was no statistical difference in *FGFR1* IHC staining between patients with and without *FGFR1* fusion ($P = 0.233$; Supplementary Table S5).

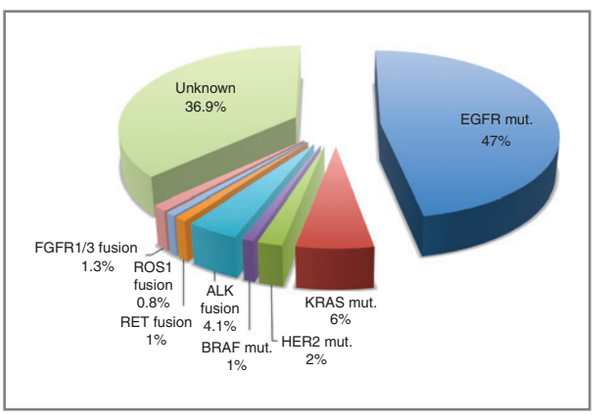

Figure 2. Spectrum of oncogenic driver mutations in 1,328 NSCLCs.

Clinical outcome

Adults who underwent surgical resection for pathologic stage I to IIIA NSCLC were eligible for survival analysis. Patients who had been treated with chemotherapy or radiotherapy before surgery were excluded. A total of 734 patients with NSCLC were included for survival analysis. The clinicopathologic characteristics of patient were listed in

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Table 3. Individual patient characteristics

Patient No.	*FGFR* fusion	Age, y	Sex	Stage	Histology	Adjuvant therapy	RFS (mo)	OS (mo)
1	*FGFR3-TACC3**	48	M	II B	SCC	Unknown	Unknown	Unknown
2	*FGFR3-TACC3*Δ	70	M	I B	SCC	Gemcitabine + cisplatin	4.6+	4.6+
3	*FGFR3-TACC3*Δ	49	M	III A	SCC	Unknown	11.4	18.4
4	*FGFR3-TACC3*°	55	M	III A	SCC	Tarceva	13.0	14.0+
5	*FGFR3-TACC3*♀	55	M	II a	SCC	Pemetrexed + carboplatin	37.0+	37.0+
6	*FGFR3-TACC3**	65	M	I B	SCC	Radiotherapy	6.0	10.5
7	*FGFR3-TACC3*♂	62	M	II B	SCC	Unknown	2.0	2.0+
8	*FGFR3-TACC3*♂	62	M	II B	SCC	Unknown	26.0+	26.0+
9	*FGFR3-TACC3*Δ	46	M	I a	SCC	Unknown	18.0+	18.0+
10	*FGFR3-TACC3*Δ	75	M	II A	AD	Navebine + cisplatin	20.3+	33.8+
11	*FGFR3-TACC3*Δ	64	M	III A	AD	Pemetrexed + cisplatin	32.2+	32.2+
12	*FGFR3-TACC3*Δ	56	M	I B	AD	Gemcitabine + cisplatin	10.7	29.1
13	*FGFR3-TACC3*Δ	52	M	I B	AD	Gemcitabine + cisplatin	25.8+	27.3+
14	*FGFR3-TACC3*Δ	62	M	II A	AD	Pemetrexed + cisplatin	12.0	13.5
15	*FGFR3-TACC3*Δ	45	F	III A	AD	Pemetrexed + cisplatin	19.5	27.0+
16	*BAG4-FGFR1*	61	M	II B	SCC	Navebine + cisplatin	22.5+	45.0+
17	*BAG4-FGFR1*	48	M	I B	SCC	Gemcitabine + cisplatin	16.0+	16.0+

Abbreviation: AD, adenocarcinoma.

*FGFR3-TACC3**, *FGFR3-TACC3* (E18:E11 INS78 bp intron from TACC3).

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FGFR3-TACC3♂, *FGFR3-TACC3* (E17:E10).

BAG4-FGFR1, *BAG4-FGFR1* (E1:E8).

Supplementary Table S6. The median follow-up time for the entire cohort was 22.0 and 26.0 months for patients with *FGFR* fusion gene. The median relapse-free survival (RFS) was 28.0 months for patients with *FGFR* fusions, 27.4 months for patients with *EGFR* mutation, 15.3 months for patients with *KRAS* mutation, 14.2 months for patients with *HER2* mutation, 7.1 months for patients with *BRAF* mutation, and 32 months for patients with *FGFR*/*EGFR*/*KRAS*/*HER2*/*BRAF* wild-type patients. The median overall survival (OS) was 34.8 months for patients with *HER2* mutation and 21.1 months for patients with *BRAF* mutation. The median OS was not reached for patients with genetic alterations in *EGFR*, *KRAS*, or *FGFR*. The outcome and adjuvant chemotherapy regiment for individual patient with *FGFR* fusions were listed in Table 3. The 2-year RFS rates were 51.4% [95% confidence interval (CI), 25.1%–77.6%] for patients with *FGFR* fusion. In comparison, patients with *EGFR* mutation, *KRAS* mutation, and *HER2* mutation had 2-year RFS rates of 55.6% (95% CI, 48.5%–62.7%), 43.2% (95% CI, 26.9%–59.5%), 44.5% (95% CI, 15.3%–73.7%), respectively. The 2-year OS rates were 83.1% (95% CI, 61.5%–100.0%) for patients with *FGFR* fusion. In comparison, patients with *EGFR*, *KRAS*, and *HER2* mutation had 2-year OS rates of 84.4% (95% CI, 79.3%–89.5%), 69.7% (95% CI, 54.2%–85.2%), and 75.2% (95% CI, 50.5%–99.9%), respectively. For NSCLCs, there was no significant difference in RFS or OS between patients with *FGFR* fusion-positive and *FGFR*-negative tumors (Fig. 3). There were no significant differences in RFS or OS for patients with *FGFR* fusions compared with patients with other driver mutations (Supplementary Fig. S3). Multivariate analysis was not feasible because of the small number of patients with *FGFR* fusions.

Discussion

FGFR fusion gene was illustrated to be a new driver for a range of cancers (12–16). The oncogenic potential of *FGFR1-TACC1*, *FGFR2-BICC1*, and *FGFR3-TACC3* was confirmed by expression of the fusion kinases in NIH3T3 fibroblasts, Rat1A fibroblasts, or astrocytes and conferred *in vivo* tumorigenesis of subcutaneous transplanted cells in immune-deficient mice (12–15). The growth of cells expressing *FGFR1/3* fusions is strongly addicted to FGFR kinase activity and were sensitive to FGFR inhibitors, such as pozopanib and PD173074, which were multitargeted kinase inhibitors that inhibit FGFR (12–15). Further prospective clinical trials are warranted to test their efficacy on treating patients with NSCLC carrying *FGFR* fusions.

In this study, we detected the *FGFR1*, *FGFR2*, and *FGFR3* gene fusions in a large scale of patients with NSCLC. Our data showed that *FGFR1/3* fusions occurred in 1.3% of patients with NSCLCs and 3.5% of patients with lung SCC. To our knowledge, this study represents the first and comprehensive analysis of the clinicopathologic features

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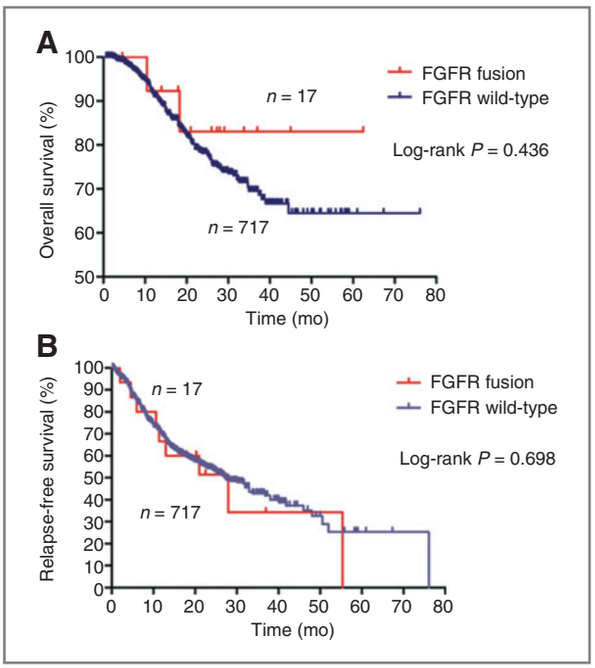

Figure 3. Kaplan–Meier survival curves for relapse-free and OS according to *FGFR* fusion or *FGFR* wild-type status in 734 patients with NSCLC. A, relapse-free survival according to *FGFR* fusion or *FGFR* wild-type. B, OS according to *FGFR* fusion or *FGFR* wild-type.

of *FGFR* fusions along with other well-identified driver mutations in a large cohort of NSCLCs. All mutation and gene fusions were mutually exclusive, indicating their role as driver mutations.

ALK, *RET*, and *ROS1* fusions were well-characterized oncogenic driver mutations in lung cancer. Our results showed that patients with *ALK*, *RET*, and *ROS1* shared very similar characteristics, associated with adenocarcinoma histology and never smoking, whereas *FGFR* fusions occur more frequently in male patients with relative larger tumor (>3 cm) and squamous histology. Knowledge of these clinical characteristics will help clinicians select those patients most likely to harbor this genetic alteration and benefit from FGFR-targeted therapy.

Current study also found that six *FGFR3-TACC3* fusions were present in lung adenocarcionma, accounting for 0.6% (6 of 1,016). Patients with lung adenocarcinomas who harbored *FGFR3-TACC3* fusion also have identifiable clinicopathologic characteristics, including relative larger tumor (>3 cm) and a solid-containing subtype, suggesting their idiographic mechanism of carcinogenesis. This finding provides a strong rationale for broadening the inclusion of FGFR-targeted therapy clinical trial to capture patients with NSCLC who carry *FGFR* fusion genes.

Although a variety of fusion partners of *FGFR* were reported by recent studies (7, 12), only one partner each for the *FGFR1* and *FGFR3* fusions (*BAG4-FGFR1* and *FGFR3-TACC3*) was found, suggesting that *TACC3* was the most common fusion partner in NSCLCs. *FGFR2* fusions were not identified in our study, indicating that *FGFR3* or *FGFR1*, other than *FGFR2* might be crucial for oncogenesis in NSCLC.

The methods for detection of gene fusions include RT-PCR, IHC, and FISH (21, 22). Nevertheless, FISH assay was not available for detecting *FGFR* rearrangements shown by previous studies because the interval of two genes (*FGFR3-TACC3* or *BAG4-FGFR1*) was too small to detect rearrangement. In current study, 14 known fusion variants of *FGFR* found in glioblastoma, cholangiocarcinoma, bladder cancer, thyroid cancer, oral cancer, breast cancer, or head and neck SCC were tested in 1,328 NSCLCs. Because RT-PCR could not be used to detect unknown fusion variants, it is possible that a few *FGFR* rearranged samples may have been missed.

For screening of *FGFR3* fusions in lung cancer, IHC with two FGFR antibodies was also tested. Expression of *FGFR3* was significantly higher in tumors with *FGFR3* fusion than in those without *FGFR3* fusion. We also demonstrated that FGFR3 expressions were detected in 11.7% (Cell Signaling Technology) and 22.3% (Abcam) of *FGFR* fusion-negative samples, suggesting that IHC with current commercial available FGFR antibodies was not an optimal tool for screening of *FGFR* fusions. However, we could not exclude the possibility that a different antibody or different techniques may produce better results.

Our results showed that *FGFR* fusions were not associated with prognosis in patients with NSCLC. The sample size of patients with *FGFR* fusions for survival analysis was too small to draw definitive conclusions and larger studies will be helpful to clarify the truly prognostic effects of *FGFR* fusions.

In summary, our data demonstrated that *FGFR1/3* fusions occurred in 1.3% of patients with NSCLCs and 3.5% of patients with lung SCC. *FGFR* fusions identified a distinct subset of NSCLC with a higher prevalence among smokers with SCC and relative larger tumor (>3 cm). Lung cancer is the most common cause of cancer-related death worldwide and with an estimated 1,608,800 new cases of lung cancer per year worldwide in 2005; this equates to more than 20,000 new affected individuals per year. Identifying even a small subpopulation of patients with gene fusions who may be potentially responsive to targeted therapy, as exemplified by the discovery of *RET* and *ROS1* fusions, may have major therapeutic relevance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Conception and design: R. Wang, L. Wang, Y. Li, J. Zhang, W. Pao, Y. Sun, H. Chen

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Wang, L. Wang, H. Hu, Y. Zhang, X. Luo, Y. Zhang, B. Pan, H. Li, J. Zhang, W. Pao, H. Chen

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