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The Impact of Cigarette Smoking on the Frequency of and Qualitative Differences in *KRAS* Mutations in Korean Patients with Lung Adenocarcinoma

Hye Ryun Kim,[1,2*] Jung Ryun Ahn,[1,2*] Jin Gu Lee,[3] Doo Hee Bang,[4] Sang-Jun Ha,[5] Yun Kyoung Hong,[6] Sun Mi Kim,[7,8] Ki Chang Nam,[9,10] Sun Young Rha,[1,2] Ross A. Soo,[11] Gregory J. Riely,[12] Joo Hang Kim,[1,2] and Byoung Chul Cho[1,2]

[1]Yonsei Cancer Center, [2]Department of Internal Medicine, and
[3]Department of Thoracic and Cardiovascular Surgery, Yonsei University College of Medicine, Seoul;
[4]Department of Chemistry, College of Science, Yonsei University, Seoul;
[5]Department of Biochemistry, College of Life Science & Biotechnology, Yonsei University, Seoul;
[6]JE UK Institute for Cancer Research, Gumi; [7]Brain Korea 21 Project for Medical Sciences,
[8]Institute for Cancer Research, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul;
[9]Department of Medical Engineering, Yonsei University College of Medicine, Seoul;
[10]Clinical Trials Centers for Medical Devices, Yonsei University Health System, Seoul, Korea;
[11]Department of Hematology-Oncology, National University Cancer Institute, National University Health System, Singapore;
[12]Memorial Sloan-Kettering Cancer Center and Weill Medical College of Cornell University, New York, NY, USA.

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Corresponding author: Dr. Byoung Chul Cho, Yonsei Cancer Center, Division of Medical Oncology, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Korea.
Tel: 82-2-2228-8126, Fax: 82-2-393-3652
E-mail: cbc1971@yuhs.ac

*Hye Ryun Kim and Jung Ryun Ahn contributed equally to this work.

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Purpose: This study was designed to determine the relationship of cigarette smoking to the frequency and qualitative differences among *KRAS* mutations in lung adenocarcinomas from Korean patients. **Materials and Methods:** Detailed smoking histories were obtained from 200 consecutively enrolled patients with lung adenocarcinoma according to a standard protocol. *EGFR* (exons 18 to 21) and *KRAS* (codons 12/13) mutations were determined via direct-sequencing. **Results:** The incidence of *KRAS* mutations was 8% (16 of 200) in patients with lung adenocarcinoma. *KRAS* mutations were found in 5.8% (7 of 120) of tumors from never-smokers, 15% (6 of 40) from former-smokers, and 7.5% (3 of 40) from current-smokers. The frequency of *KRAS* mutations did not differ significantly according to smoking history (p=0.435). Never-smokers were significantly more likely than former or current smokers to have a transition mutation (G→A or C→T) rather than a transversion mutation (G→T or G→C) that is known to be smoking-related (p=0.011). In a Cox regression model, the adjusted hazard ratios for the risk of progression with epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) were 0.24 (95% CI, 0.14-0.42; p<0.001) for the *EGFR* mutation and 1.27 (95% CI, 0.58-2.79; p=0.537) for the *KRAS* mutation. **Conclusion:** Cigarette smoking did not influence the frequency of *KRAS* mutations in lung adenocarcinomas in Korean patients, but influenced qualitative differences in the *KRAS* mutations.

Key Words: *EGFR*, *KRAS*, pulmonary adenocarcinoma, cigarette smoking, EGFR-tyrosine kinase inhibitors

INTRODUCTION

Lung cancer is one of the most common malignancies and is a leading cause of cancer-related mortality worldwide.[1,2] Recent development and approval of the epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, have significantly changed treatment outcomes in advanced non-small cell lung cancer (NSCLC).[3] Patients with adenocarcinoma who are of Asian origin, female gender, and with no history of smoking predict a strong response to EGFR-TKIs.[4] In addition, molecular studies show a number of biomarkers associated with tumor sensitivity to EGFR-TKIs.[5-9] The presence of *EGFR* mutations, in particular, is associated with dramatic response to EGFR-TKIs.[5-7,9,10] On the other hand, somatic mutations of the *KRAS* oncogene may predict poor EGFR-TKI responsiveness.[3,11-17]

The *RAS* gene subfamily encodes a group of guanosine triphosphate-binding proteins, which are essential components of the *EGFR* signaling cascade and play important roles in tumor pathogenesis.[18,19] Single nucleotide mutations in *KRAS* codons 12 and 13 compromise guanosine triphosphatase (GTPase) activity.[19,20] Such mutations may not only impair the intrinsic *RAS* GTPase activity, but also confer resistance to GTPase-activating proteins. Consequently, *RAS* accumulates in its active GTP-bound state, resulting in constitutively activated *RAS* signaling.[21] *KRAS* mutations are frequently observed in lung adenocarcinomas and may be smoking-related, while *KRAS* mutations are uncommon in squamous cell lung carcinomas and lung cancers in never-smokers.[18,22,23] Interestingly, *KRAS* mutations occur more commonly in the lung tumors of Caucasian patients than in those of East Asians.[21]

Since *KRAS* mutations are common in NSCLC and cigarette smoking is a frequent cause of NSCLC, *KRAS* mutations are hypothesized to be related to tobacco exposure.[18] However, studies to test the association between cigarette smoking and *KRAS* mutation often lack detailed patient smoking histories and include relatively small numbers of never-smokers.

The validity of the *KRAS* mutation as a predictive biomarker for lung cancer response to EGFR-TKIs remains uncertain. Several reports support an association between the presence of *KRAS* mutation and poor response to EGFR-TKIs.[11-13,15,16,24,25] On the other hand, results of the IRESSA Non-Small-Cell-Lung Cancer Trials Evaluating Response and Survival Against Taxotere trial show no difference in overall survival (OS), progression-free survival (PFS), or response rate according to *KRAS* mutation status.[10,26]

Few studies provide detailed correlations of *KRAS* mutations with smoking history or treatment outcome following treatment with EGFR-TKIs. We, therefore, conducted this study to determine the relationship of cigarette smoking with the frequency and qualitative differences in *KRAS* mutations in the lung adenocarcinomas of Korean patients. In addition, based on the concurrent mutational analysis, we evaluated the power of *KRAS* mutation status to predict treatment outcome with EGFR-TKIs in these patients.

MATERIALS AND METHODS

Study population and data collection

For this study, we enrolled 200 consecutive patients who had lung adenocarcinomas that were newly diagnosed and histologically confirmed between October 2007 and April 2010 at the Yonsei Cancer Center in Seoul, Korea and who were available for genetic analysis. The tumor histology was classified using the World Health Organization criteria.[27] Detailed smoking histories were prospectively obtained from these 200 patients with NSCLC according to a standard protocol that included the following questions:[28] Have you smoked more than 100 cigarettes in your life? Are you currently smoking? How many years have you been a regular smoker; and on average, how many cigarettes did you smoke per day? The smoking questionnaire was administered by a medical oncologist. Based on their smoking status, patients were categorized as never-smokers (<100 cigarettes in their lifetime), former-smokers (quit ≥1 year ago), or current-smokers (quit <1 year ago). Pack-years of smoking were defined as [(average number of cigarettes per day/20)×years of smoking]. For all patients, medical records were reviewed to extract data based on their clinicopathological characteristics. For patients with metastatic disease, we examined treatment regimens, overall response rates, and survival outcomes (PFS, OS). Clinical responses were assessed every two cycles using computerized tomography and were classified using the Response Evaluation Criteria in Solid Tumor (RECIST version 1.0).[29] PFS was measured from the first day of treatment with EGFR-TKI to progression or death, while OS was measured from the date of treatment with EGFR-TKI until the date of death. Patients were censored on July 31, 2010, if alive and progression-free. Patients

with no known date of death were censored on the date of their final follow-up. This study was approved by the Severance Hospital Institutional Review Board. All patients signed a written informed consent for genetic analysis.

EGFR and *KRAS* mutation analysis

Nucleotide sequencing of the kinase domain of *EGFR* (exons 18 to 21) was performed using nested polymerase chain reaction amplification of the individual exons.[17] The sequencing protocol has been previously described.[13,28] Specific mutations in *KRAS* exon 2 (codons 12 and 13) were identified from published data.[13,28]

Statistical analysis

Data were summarized using standard descriptive statistics. Significant differences in the variables between genotypes were tested using the χ^2 test, Fisher's exact test, and t-tests where appropriate. The Kaplan-Meier method was used to estimate PFS and OS, and the differences between genotypes were compared using the log-rank test. The adjusted hazard ratios (AHRs) for the risk of progression or death with treatment were compared between genotypes using a Cox regression model that included age, gender, smoking history, and performance status as independent variables. All *p* values were two-sided.

RESULTS

KRAS mutation and smoking history

This entire cohort included 93 men and 107 women patients with a median age of 58 years (range: 28-84). All histologic types were adenocarcinomas (Table 1). Based on smoking history, patients were classified into three groups: never-smokers (n=120, 60%), former smokers (n=40, 20%), and current smokers (n=40, 20%) (Table 1).

Among the 200 patients, 87 (43.5%) had *EGFR* mutations and 16 (8%) had *KRAS* mutations in codons 12 (n=

Table 1. Patient Characteristics

Characteristic	n	*KRAS* mutation (%)	*p* value*
Age, yrs			0.188
≤60	112	6 (5.4)	
>60	88	10 (11.4)	
Median age (range)	58 (28-84)	61.5 (44-74)	
Sex			0.770
Women	107	8 (7.5)	
Men	93	8 (8.6)	
ECOG PS			NS
0-1	173	15 (8.6)	
2	22	1 (4.5)	
Histology			
Adenocarcinoma	200	16 (8.0)	
Stage‡			NS
I-II	60	5 (8.3)	
III-IV	140	11 (7.9)	
Smoking status			0.167†
Never§	120	7 (5.8)	
Former‖	40	6 (15.0)	
Current¶	40	3 (7.5)	
EGFR			0.002
Wild-type	113	15 (13.3)	
Mutant	87	1 (1.1)	

NS, not significant; *EGFR*, epidermal growth factor receptor; ECOG PS, Eastern Cooperative Oncology Group Performance Status.
*According to Pearson's χ^2 or Fisher's exact test.
†Never versus others.
‡Stage at the time of initial diagnosis.
§Never-smokers had smoked fewer than 100 cigarettes.
‖Former smokers had previously smoked cigarettes but quit smoking more than one year prior to diagnosis of lung cancer.
¶Current smokers had quit less than one year ago.

Supplementary Table 1. Summarized Treatment Outcome with EGFR-TKI of Individual Patients with Metastatic Lung Adenocarcinoma (n=96)

Patient no.	Age	Sex	Stage	*EGFR* mutation	*KRAS* mutation	Smoking status	EGFR TKI	Best response	PFS* (months)	OS† (months)	Alive
1	61	M	IV	Negative	Gly12Ser	Former	G	PD	1.0	5.9	No
2	67	M	IV	Negative	Gly12Val	Former	G	PD	1.3	1.3	No
3	50	F	IV	Negative	Gly12Ser	Never	G	PD	1.0	15.9	No
4	58	M	IIIB	Negative	Gly12Cys	Former	E	PD	1.0	1.1	No
5	44	M	IIIB	Negative	Gly12Val	Current	E	PR	32.0	45.8	Yes
6	70	F	IIIB	Negative	Gly12Asp	Never	G	SD	7.0	9.3	No
7	61	F	IIIB	Negative	Gly12Asp	Never	E	SD	1.6	25.6	No
8	58	F	IB	Negative	Gly>Asp	Never	G	PD	2.2	9.3	No
9	56	M	IV	20 (T790M)/ 21 (L858R)	Negative	Never	G	PD	1.0	2.6	Yes
10	61	M	IV	His773Tyr/ 21 (L858R)	Negative	Former	G	PD	2.0	5.9	No
11	69	F	IV	21 (L858R)	Negative	Never	G	PD	0.3	0.4	No
12	46	M	IV	19 (del)	Negative	Never	G	PD	0.9	1.1	No
13	59	M	IV	19 (del)	Negative	Former	G	PR	1.5	1.5	Yes
14	68	F	IV	21 (L858R)	Negative	Never	G	PR	14.0	14.0	Yes
15	48	F	IV	21 (L858R)	Negative	Never	G	PR	46.0	47.5	Yes
16	54	F	IV	19 (del)	Negative	Never	G	PR	17.0	22.5	Yes
17	66	M	IV	21 (L858R)	Negative	Former	E	PR	10.8	13.3	No
18	47	F	IV	21 (L858R)	Negative	Never	E	PR	14.1	22.3	Yes
19	67	F	IV	19 (del)	Negative	Never	E	PR	6.5	8.3	No
20	68	F	IV	19 (del)	Negative	Never	E	PR	8.0	13.8	No
21	52	M	IV	21 (L858R)	Negative	Current	E	PR	7.0	11.0	No
22	50	M	IV	19 (del)	Negative	Never	G	PR	12.3	26.8	No
23	58	F	IV	19 (del)	Negative	Never	G	PR	23.4	33.3	No
24	46	F	IV	19 (del)	Negative	Never	G	SD	10.5	10.5	Yes
25	64	M	IV	21 (L858R)	Negative	Former	G	SD	9.6	9.7	Yes
26	50	M	IV	21 (L858R)	Negative	Current	G	SD	3.0	3.0	Yes
27	58	F	IV	21 (L858R)	Negative	Never	G	SD	22.5	22.5	Yes
28	42	F	IV	21 (L858R)	Negative	Never	G	SD	3.2	3.2	Yes
29	41	M	IV	19 (del)	Negative	Former	E	SD	1.3	1.3	Yes
30	67	F	IV	19 (del)	Negative	Never	G	SD	10.0	12.6	No
31	64	F	IV	19 (del)	Negative	Current	G	SD	1.9	2.6	No
32	54	F	IV	19 (del)	Negative	Never	E	SD	10.3	22.5	No
33	51	M	IV	19 (del)	Negative	Former	E	SD	8.7	25.2	Yes
34	47	F	IV	21 (L858R)	Negative	Never	G	SD	8.5	10.3	No
35	58	M	IV	19 (del)	Negative	Current	G	SD	14.1	34.1	No
36	43	F	IV	19 (del)	Negative	Never	G	SD	23.9	52.5	Yes
37	48	F	IV	21 (L858R)	Negative	Never	G	SD	13.7	36.4	No
38	48	F	IV	21 (L858R)	Negative	Never	G	SD	13.7	36.4	No
39	56	F	IV	19 (del)	Negative	Never	G	SD	19.3	24.8	No
40	61	F	IV	19 (del)	Negative	Never	G	SD	13.6	13.6	No
41	37	F	IIIB	19 (del)	Negative	Never	G	PR	8.7	30.7	No
42	50	F	IIIB	19 (del)	Negative	Never	G	SD	3.2	16.2	No
43	73	M	IIB	19 (del)	Negative	Never	G	SD	9.1	9.2	Yes
44	30	M	IIA	19 (del)	Negative	Former	G	PD	19.7	34.5	Yes
45	71	M	IB	19 (del)	Negative	Former	E	SD	11.2	12.0	Yes
46	54	M	IB	21 (L858R)	Negative	Never	G	SD	34.5	48.6	Yes
47	65	F	IA	19 (del)	Negative	Never	E	PR	11.4	11.4	Yes
48	44	F	IA	21 (L858R)	Negative	Never	E	SD	12.8	41.5	No

Supplementary Table 1. Continued

Patient no.	Age	Sex	Stage	*EGFR* mutation	*KRAS* mutation	Smoking status	EGFR TKI	Best response	PFS* (months)	OS† (months)	Alive
49	60	F	IA	19 (del)	Negative	Never	G	SD	7.7	13.6	No
50	45	F	IV	Negative	Negative	Never	G	PD	0.9	2.7	Yes
51	53	M	IV	Negative	Negative	Current	G	PD	0.5	0.6	No
52	39	M	IV	Negative	Negative	Never	E	PD	1.1	1.1	No
53	70	M	IV	Negative	Negative	Current	G	PD	0.9	2.1	No
54	56	M	IV	Negative	Negative	Current	E	PD	1.1	3.9	No
55	62	M	IV	Negative	Negative	Current	E	PD	1.0	13.8	No
56	70	M	IV	Negative	Negative	Former	G	PD	0.2	1.0	No
57	57	M	IV	Negative	Negative	Current	E	PD	0.4	0.5	No
58	50	F	IV	Negative	Negative	Never	E	PD	0.9	0.9	No
59	60	F	IV	Negative	Negative	Never	E	PD	1.3	5.1	No
60	45	M	IV	Negative	Negative	Former	E	PD	0.2	0.3	No
61	56	F	IV	Negative	Negative	Never	E	PD	0.6	28.4	Yes
62	30	F	IV	Negative	Negative	Never	E	PD	2.1	4.8	No
63	37	F	IV	Negative	Negative	Never	G	PD	0.6	4.0	No
64	55	F	IV	Negative	Negative	Never	G	PD	3.7	5.8	No
65	74	M	IV	Negative	Negative	Current	G	PD	1.0	3.9	No
66	68	M	IV	Negative	Negative	Former	G	PD	2.1	4.8	No
67	65	M	IV	Negative	Negative	Former	E	PD	1.8	4.2	No
68	55	M	IV	Negative	Negative	Former	G	PD	1.0	12.5	No
69	75	M	IV	Negative	Negative	Current	G	PR	22.6	41.3	No
70	54	M	IV	Negative	Negative	Current	E	PR	1.8	15.5	No
71	73	F	IV	Negative	Negative	Never	E	PR	0.4	0.6	No
72	50	M	IV	Negative	Negative	Never	G	PR	7.2	18.0	No
73	54	M	IV	Negative	Negative	Former	E	SD	0.9	1.0	Yes
74	56	F	IV	Negative	Negative	Never	G	SD	2.6	2.7	Yes
75	50	M	IV	Negative	Negative	Current	G	SD	7.4	7.9	Yes
76	72	M	IV	Negative	Negative	Never	E	SD	6.9	14.9	No
77	58	F	IV	Negative	Negative	Former	E	SD	1.2	8.3	No
78	35	F	IV	Negative	Negative	Never	G	SD	6.4	17.6	No
79	75	M	IIIB	Negative	Negative	Former	G	PD	1.0	4.0	Yes
80	36	M	IIIB	Negative	Negative	Current	E	PD	3.0	10.8	No
81	67	M	IIIB	Negative	Negative	Former	G	PD	3.1	8.0	No
82	52	F	IIIB	Negative	Negative	Never	G	PD	1.2	12.6	No
83	67	F	IIIB	Negative	Negative	Never	G	SD	4.5	21.0	Yes
84	54	F	IIIB	Negative	Negative	Never	G	SD	3.3	11.9	No
85	64	M	IIIB	negative	Negative	Current	E	SD	4.5	39.8	Yes
86	59	F	IIIA	Negative	Negative	Never	G	PD	1.0	6.4	No
87	59	F	IIIA	Negative	Negative	Never	G	PR	3.9	5.1	No
88	36	F	IIIA	Negative	Negative	Never	G	SD	14.0	14.1	Yes
89	52	F	IIIA	Negative	Negative	Never	E	SD	8.0	14.2	Yes
90	57	M	IIIA	Negative	Negative	Never	G	SD	2.4	2.6	No
91	51	F	IIIA	Negative	Negative	Never	G	SD	6.6	35.6	No
92	56	M	IIIA	Negative	Negative	Never	G	SD	4.3	36.1	No
93	54	M	IIB	Negative	Negative	Never	G	PD	2.0	2.2	No
94	66	M	IB	Negative	Negative	Former	E	PD	0.3	0.5	No
95	68	M	IB	Negative	Negative	Former	G	SD	5.7	8.5	No
96	52	M	IB	Negative	Negative	Current	G	SD	2.1	12.0	No

PFS, progression free survival; OS, overall survival; PR, partial response; SD, stable disease; PD, progressive disease; G, gefitinib; E, erolotinib; EGFR, epiderman growth factor receptor; TKI, tyrosine kinase inhibitor.

*PFS was measured from the first day of treatment with EGFR-TKI to the progression or death.

†OS was measured from the date of treatment with EGFR-TKI until the date of death.

14) or 13 (n=2). One patient had both an *EGFR* mutation (exon 19, del 2235-2249) and a *KRAS* mutation (Gly12Cys, GGT→TGT). Patients with a *KRAS* mutation and those with wild-type *KRAS* (*WT*) did not differ in baseline characteristics including age, gender, Eastern Cooperative Oncology Group (ECOG) performance status, histology, stage at initial diagnosis, and smoking history (Table 1). Presence of the *KRAS* mutation was not significantly related to smoking history (Mantel-Haenszel χ^2 test, p= 0.435). *KRAS* mutations were identified in 5.8% (7/120) of never-smokers, 15% (6/40) of former smokers, and 7.5% (3/40) of current smokers (Fig. 1). Moreover, age at first cigarette, total pack-years, total smoke-years, and smoke free-years were not correlated with *KRAS* mutations (Supplementary Fig. 1).

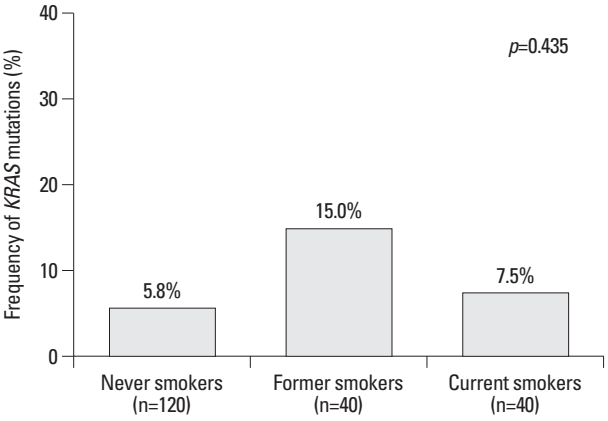
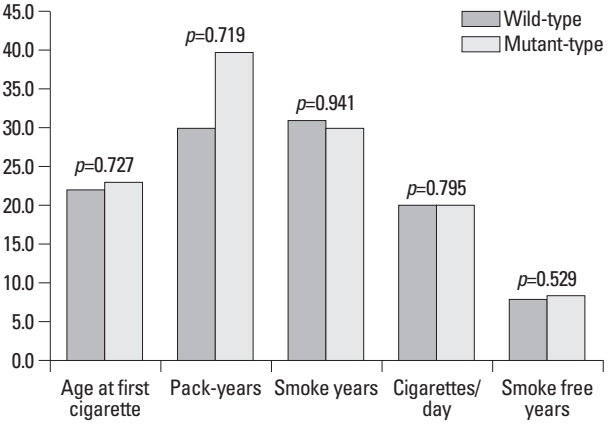


Fig. 1. Frequency of *KRAS* mutation according to smoking history.



Supplementary Fig. 1. Comparison of median values for various factors regarding cigarette-smoking according to *KRAS* mutation status. The number of y-axis represents the number according to the variables in x-axis (age at first cigarette, pack-years, smoke years, cigarettes per day, and smoke free year), respectively.

Never-smokers were significantly more likely than former- or current smokers to have a transition mutation (substitution of a purine for a purine, e.g., G→A or a pyrimidine for a pyrimidine, C→T) than a transversion mutation (substitution of a purine for a pyrimidine or conversely, G→T or G→C), which are known to be smoking-related (p= 0.011) (Table 2).

Treatment outcome with *EGFR* tyrosine kinase inhibitors

Of 153 patients with advanced lung adenocarcinoma, 97 received EGFR-TKIs (Supplementary Table 1). Among them, one patient had both *EGFR* and *KRAS* mutations detected and received a combination treatment of erlotinib and sorafenib in a clinical trial. This patient showed a partial response (PR) lasting more than 15 months. Among the 41 patients with *EGFR* mutations, 25 (61.0%) showed a clinical response to EGFR-TKIs, 11 (26.8%) had stable disease (SD), and 5 patients (12.2%) had progressive disease (PD). In the 8 patients with *KRAS* mutations, one (12.5%) showed a clinical response to EGFR-TKIs, 2 (25.0%) had SD, and 5 (62.5%) had PD on EGFR-TKIs (Table 3). In 47 patients with wild-type *EGFR* and *KRAS* (*WT/WT*), 5 (10.6%) had PR, 16 patients (34.0%) had SD, and 26 patients (55.3%) had PD on EGFR-TKIs. As a group, patients with *KRAS* mutations had a significantly poorer clinical response to EGFR-TKIs than those who had *EGFR* mutations (12.5% vs. 61.0%, p=0.004), but did not differ in clinical response from those with *WT/WT* (10.6% vs.12.5%, p=0.876).

At the time of analysis, the median follow-up duration of

Table 2. Types of *KRAS* Mutations According to Smoking History

Type of *KRAS* mutation	Nucleotide	Never-smoker	Ever-smoker	*p* value
Transition				0.011*
G13D	GGC→GAC	0	1	
G12D	GGT→GAT	4	1	
G12S	GGT→AGT	2	1	
Transversion				
G12C	GGT→TGT	1	2	
G12V	GGT→GTT	0	3	
G13C	GGC→TGC	0	1	

*Fisher's exact test, transition versus transversion.

Table 3. Characteristics and TKI Responses of Nine Lung Cancer Patients with *KRAS* Mutations

No.	Sex	Smoking status	Type of *KRAS* mutation	*EGFR* mutation	Type of TKI	Best response	PFS* (months)	OS† (months)
1	M	Former	Transition	No	G	PD	1.0	5.9
2	M	Former	Transversion	No	G	PD	1.3	1.3
3	F	Never	Transition	No	G	PD	1.0	15.9
4	M	Former	Transversion	No	E	PD	1.0	1.1
5	M	Current	Transversion	No	E	PR	32.0	45.8
6	F	Never	Transition	No	G	SD	7.0	9.3
7	F	Never	Transition	No	G	SD	1.6	25.6
8	F	Never	Transition	No	G	PD	2.2	9.3

TKI, tyrosine kinase inhibitor; PFS, progression-free survival; OS, overall survival; PR, partial response; SD, stable disease; PD, progressive disease; G, gefitinib; E, erlotinib; *EGFR*, epidermal growth factor receptor.
*PFS was measured from the first day of treatment with EGFR-TKI to the date of progression or death.
†OS was measured from the date of treatment with EGFR-TKI until the date of death.

Table 4. Prognostic Significance of Gene Mutations in Metastatic Lung Adenocarcinoma Treated with EGFR TKIs

Genotype	PFS for EGFR TKIs			OS*		
	No. of mutant/ wild-type	AHR† (95% CI)	*p* value	No. of mutant/ wild-type	AHR† (95% CI)	*p* value
EGFR (+ v -)	41/55	0.246 (0.144-0.421)	<0.001	41/55	0.453 (0.260-0.787)	0.005
KRAS (+ v -)	8/88	1.278 (0.586-2.790)	0.537	8/88	1.274 (0.571-2.845)	0.554

EGFR, epidermal growth factor receptor; TKIs, tyrosine kinase inhibitors; AHR, adjusted hazard ratio; 95% CI, 95% confidence interval; OS, overall survival; PFS, progression-free survival.
*Overall survival was measured from the initiation of EGFR-TKIs until death.
†Adjusted for age, gender, smoking history and performance status

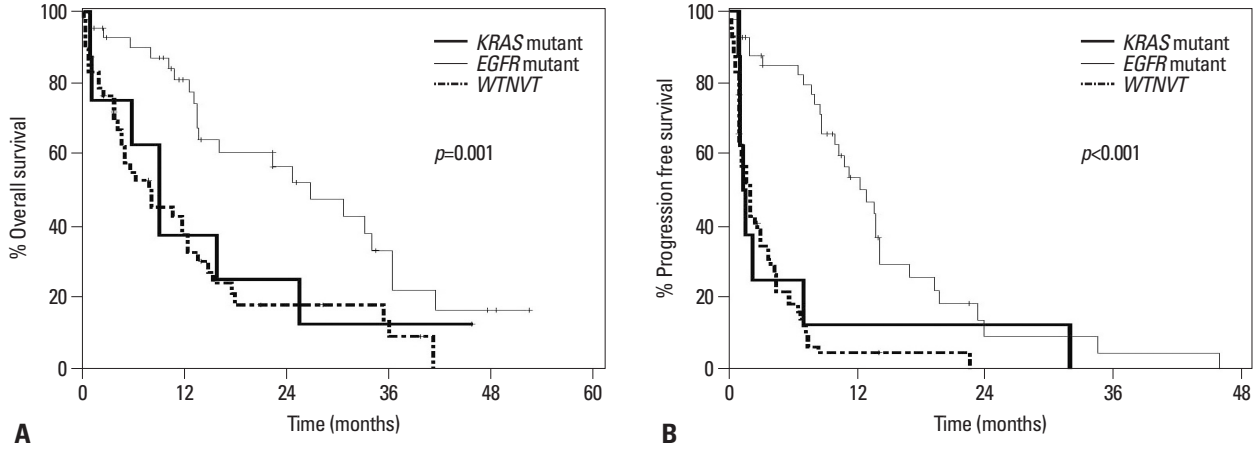


Fig. 2. Kaplan-Meier survival plots for patients with three tumor genotypes in metastatic lung adenocarcinoma treated with EGFR tyrosine kinase inhibitors (TKIs): *EGFR* mutation (n=41), *KRAS* mutation (n=8), and *WT/WT* (n=47). (A) Median OS with EGFR TKI treatment (26.7 months for *EGFR* mutation vs. 8.2 months for *WT/WT* vs. 9.2 months for *KRAS* mutant, p=0.001). (B) Median PFS (12.8 months for *EGFR* mutation vs. 1.2 months for *KRAS* mutation vs. 1.9 months for *WT/WT*, p<0.001).

patients with advanced lung adenocarcinoma was 17.4 months. At the time of analysis, 103 (51.5%) of the 200 patients were still alive. In this survival outcome analysis, we excluded one patient who had both *EGFR* and *KRAS* mutations and who was treated with erlotinib/sorafenib because this dual inhibition of the *EGFR* and *vascular endothelial growth factor* (*VEGF*) pathways could influence survival outcome and cause a bias. Thus, the final analysis of survival outcome included 96 patients.

The three genotypes according to the presence of *EGFR* or *KRAS* mutation showed significant differences in median OS with EGFR-TKI treatment (26.7 months for the *EGFR* mutant, 8.2 months for *WT/WT*, and 9.2 months for the *KRAS* mutant, p=0.001) (Fig. 2A). In a Cox regression model adjusted for age, gender, smoking history and performance status, the AHRs for OS were 0.453 (95% CI, 0.260-

0.787; p=0.005) for the *EGFR* mutant and 1.274 (95% CI, 0.571-2.842, p=0.554) for the *KRAS* mutant (Table 4).

The three genotypes differed significantly in median PFS (12.8 months for the *EGFR* mutant vs. 1.2 months for the *KRAS* mutant vs. 1.9 months for *WT/WT*, p<0.001). In a pair-wise comparison, the *EGFR* mutant showed a significantly longer median PFS than did the *KRAS* mutant or the *WT/WT*. The *KRAS* mutant and *WT/WT* did not differ significantly (p=0.588) (Fig. 2B). In a Cox regression model adjusted for age, gender, smoking history and performance status, the AHRs for the risk of disease progression with EGFR-TKI treatment was 0.246 (95% CI, 0.144-0.421; p< 0.001) for the *EGFR* mutation and 1.278 (95% CI, 0.586-2.790; p=0.537) for the *KRAS* mutation (Table 4). These data identified the *EGFR* mutation as an independent positive predictive factor for treatment outcome with EGFR-TKIs and showed that the *KRAS* mutation did not predict treatment outcome with EGFR-TKIs. The outcome is totally driven by presence or absence of *EGFR* mutation.

DISCUSSION

To our best knowledge, this is the largest comprehensive analysis of the *KRAS* mutation and its association with smoking history performed to date in East Asian patients with lung adenocarcinomas. The main finding of our study was a lower incidence of the *KRAS* mutation in these tumors among our Korean study group compared to that reported for Western populations. Interestingly, cigarette smoking did not influence the frequency of *KRAS* mutation, but rather the type of *KRAS* mutation; and *KRAS* mutation did not independently predict outcome of EGFR-TKI therapy.

The low frequency of *KRAS* mutation in our study (8%, 16 of 200) is in good agreement with previous findings of *KRAS* mutation of less than 10% in East Asian patients with lung adenocarcinoma, compared with the recorded 30% or more among Caucasian patients.[24,30-35] This difference might be explained by ethnic and environmental factors, in addition to reported differences in gender and smoking status distributions.[34]

The frequency of *KRAS* mutation did not differ according to smoking status in our study. In addition, the specific characteristics of smoking such as cumulative dose (pack-years), age at first exposure, and smoke-free years did not correlate with *KRAS* mutation frequency. A strong association between *KRAS* mutation and cigarette smoking has been established.[22,23] However, careful scrutiny of these studies reveals that they included relatively small numbers of never-smokers and neglected to present detailed smoking histories. In a study of lung adenocarcinomas from almost 500 patients (17% never-smokers), Riely, et al.[28] reported the presence of a *KRAS* mutation in 15% of tumors from never-smokers compared to 22% in the group overall, showing that smoking history does not clearly predict *KRAS* mutation status. In contrast, data from a recent meta-analysis show a significantly higher *KRAS* mutation frequency among current- or former smokers compared to that of never-smokers (26% vs. 6%, p<0.01).[3] In our study, careful interpretation will be required because the lack of statistical significance might be caused by insufficient sample size. The association of *KRAS* mutation (i.e., presence of the mutation in the tumor) with smoking history, which includes cumulative smoking dose, age at first exposure, smoke-free years, and other factors, awaits further observation and analysis of large populations with detailed smoking histories.

Importantly, *KRAS* mutations observed in never-smokers are significantly more likely to be transition mutations than those in current- and former smokers, which is consistent with previous data.[28] Similarly, transition mutations in *TP53* are more common in never-smoker patients than transversions. This prevalence for transversions in *TP53* may identify a molecular signature for the action of specific carcinogen(s) in cigarette smoke.[28,36] Thus, the qualitative differences in *KRAS* mutations in lung tumors from never-smokers may be related to intrinsic tumorigenesis rather than to exposure to extrinsic carcinogens such as second-hand tobacco smoke.

The role of *KRAS* mutation in predicting survival with EGFR-TKIs treatment remains unclear.[3,19] Although most studies show that a *KRAS* mutation predicts a poor response to EGFR-TKIs, these data do not strongly support an association between *KRAS* mutation and survival outcome because the reported treatment outcomes are not survival outcome such as OS or PFS, but response rate.[3,11,13,15-17] Additionally, because *EGFR* and *KRAS* mutations are mutually exclusive, patients with *KRAS* mutations do not harbor *EGFR* mutations, whereas those with wild-type *KRAS* may potentially harbor *EGFR* mutations. Given the extreme sensitivity of *EGFR* mutant tumors to EGFR-TKIs, even a small proportion of patients with *EGFR* mutation in a cohort with *KRAS* wild-type tumors could confound or bias the association between mutation type and treatment outcome with EGFR-TKIs. To clarify the prognostic significance of *KRAS* mutation for these treatment outcomes, concurrent mutational

analysis of *EGFR* and *KRAS* is mandatory.

To overcome these problems, we compared the PFS and OS of EGFR-TKIs treatment among three tumor groups, namely those with the *EGFR* mutant, the *KRAS* mutant, and *WT/WT*, with a concurrent analysis of *EGFR* and *KRAS* mutations. In particular, we compared the treatment outcomes between patients with *KRAS*-mutant and *WT/WT* tumors to detect clinically relevant differences in EGFR-TKI effectiveness. As expected, patients with the *EGFR* mutations showed significantly longer median OS and PFS than did those with *KRAS*-mutant and *WT/WT* tumors. However, those with *KRAS*-mutant and *WT/WT* tumors did not differ significantly in outcome even though there is a limitation by small number of *KRAS* mutation positive patients (n=8). This result is consistent with report by Jackman, et al.[8] that the presence of the *KRAS* mutation should not be used as a predictive biomarker to exclude patients from EGFR-TKI treatment.

Although use of the *KRAS* genotype as a basis for treatment with *EGFR*-directed agents is controversial, it may be used to guide appropriate targeting of other treatments. Evidence suggests, for example, that dual inhibition of *VEGF* and *EGFR* pathways may overcome primary and acquired resistance to EGFR-TKIs.[37] *In vivo* xenograft models, including some with *KRAS* mutations, show a stronger anti-tumor response to a dual blockade of *VEGF/EGFR* pathways, either by vandetanib or a combination of bevacizumab and erlotinib, than to erlotinib or gefitinib alone.[37] In these models, EGFR-TKI resistance is associated with increased tumor- and host-derived *VEGF*.[37] Thus, a *VEGFR/EGFR* dual blockade may plausibly be used to overcome EGFR-TKI resistance in NSCLC with the *KRAS* mutation.

In conclusion, this study found no differences in *KRAS* mutation frequencies among lung adenocarcinomas according to cigarette-smoking status, but did show qualitative differences, in that never-smokers were significantly more likely than current or former smokers to have transition mutations rather than transversions. Furthermore, even though testing for *KRAS* mutation in lung adenocarcinoma may have little use in the decision to treat with EGFR-TKIs, it may guide the targeting of individualized treatments, which might include a dual blockade of *EGFR* and *VEGF* pathways and use of *RAF* or *MEK* inhibitors.

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