Somatic Mutations in Epidermal Growth Factor Receptor Signaling Pathway Genes in Non-small Cell Lung Cancers

Shin Yup Lee, MD, Min Jung Kim, MS,† Guang Jin, MS,†‡ Seung Soo Yoo, MD,* Ji Young Park, MD,§ Jin Eun Choi, PhD,† Hyo Sung Jeon, PhD,† Sukki Cho, MD,‖ Eung Bae Lee, MD,‖ Seung Ick Cha, MD,* Tae-In Park, MD,§ Chang Ho Kim, MD,* Tae Hoon Jung, MD,* and Jae Yong Park, MD, PhD*†*

Introduction: Epidermal growth factor receptor (EGFR) signaling pathway plays a crucial role in the development and progression of lung cancer. We searched for mutations of EGFR pathway genes in non-small cell lung cancers (NSCLCs) and analyzed their relationship with clinicopathologic features.

Methods: Mutations of *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *KRAS*, *NRAS*, *BRAF*, *PTEN*, *PIK3CA*, *LKB1,* and *AKT1* genes were determined by direct sequencing in 173 surgically resected NSCLCs—56 squamous cell carcinomas (SCCs) and 117 adenocarcinomas (ACs).

Results: Of the 173 NSCLCs, a total of 65 mutations were detected in 63 (36.4%) tumors—10 (17.9%) in SCCs and 53 (45.3%) in ACs. Mutations in EGFR pathway genes were significantly more frequent in women and ACs than in women and SCCs ($p = 0.02$ and $p < 0.001$, respectively). The mutations occurred in a mutually exclusive pattern. When the genes were divided into three subgroups according to their roles in the signaling cascade, mutations in the *EGFR/ERBB2* and *KRAS/BRAF* genes were more frequent in ACs than in SCCs ($p < 0.001$ and $p = 0.01$, respectively). In marked contrast, mutations in the *PIK3CA/PTEN* were more frequent in SCCs than in ACs ($p = 0.002$). Furthermore, mutations in the *PIK3CA/PTEN* genes were more frequent in smokers ($p = 0.04$).

Discussion: Our study demonstrates that mutations in each part of the EGFR pathway were associated with different clinicopathologic features in patients with NSCLCs.

Key Words: Epidermal growth factor receptor (EGFR) pathway, Mutation, Lung cancer.

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Departments of *Internal Medicine and †Biochemistry, School of Medicine, Kyungpook National University, Daegu, Republic of Korea; ‡Department of Pharmacology, Yanbian University School of Basic Medicine, Yanji, Jilin Province, China; and Departments of §Pathology and ‖Thoracic Surgery, School of Medicine, Kyungpook National University, Daegu, Republic of Korea.

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Address for correspondence: Jae Yong Park, MD, PhD, Department of Internal Medicine, Kyungpook National University Hospital, Samduk 2Ga 50, Daegu 700-412, Republic of Korea. E-mail: jaeyong@knu.ac.kr

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Epidermal growth factor receptor (EGFR) signaling pathway plays a crucial role in many carcinogenic processes such as proliferation, angiogenesis, invasion, and metastasis, and resistance to apoptosis.[1,2] RAS/RAF/mitogen activated protein kinase (MAPK), phosphatidylinositol 3-kinase (P13K)/AKT, signal transducer and activator of transcription, v-src sarcoma viral oncogene homolog (SRC)/focal adhesion kinase (FAK), and phospholipase C pathways are known downstream signaling cascades transducing EGFR activation signals, with RAS/RAF/MAPK and PI3K/AKT being the two major pathways.[3,4]

Because deregulation of EGFR pathway genes has been observed frequently in various types of tumors, including non-small cell lung cancer (NSCLC), the development of targeted agents for lung cancer therapy has focused mainly on EGFR and its downstream signaling networks.[4] Mutations in the tyrosine kinase (TK) domain of the *EGFR* gene were identified in a subgroup of lung cancer having a good response to EGFR TK inhibitors.[5,6] In addition, alterations of several genes in the EGFR pathway have been reported to be associated with a clinical response to EGFR TK inhibitors.[7–10] A comprehensive understanding of mutations in EGFR signaling pathway genes may, therefore, lead to optimized therapeutic approaches to lung cancer.[11]

It has been reported that the prevalence of mutations in several EGFR pathway genes, such as *EGFR*, *KRAS*, and *LKB1* differed among various ethnic groups,[12,13] which may reflect the difference in genetic and epidemiologic characteristics of lung cancer among them. Furthermore, there have been a limited number of studies that comprehensively investigated mutations in EGFR pathway genes, although individual genes in this pathway have been widely investigated. In this study, we searched for mutations in EGFR signaling pathway genes in Korean patients with NSCLCs, combining our previously reported data on *PTEN* and *LKB1* mutations in lung cancers,[14,15] correlating the results with clinicopathologic features.

PATIENTS AND METHODS

Patients and Tissue Samples

After obtaining approval from the institutional review board and the patients' written informed consent, primary lung tumors and matching nonmalignant lung tissues and

TABLE 1. Patient Characteristics

Variables	Total	SCC	AC	*p*
Sample size	173	56	117	
Age (yr), mean ± SD	62.7 ± 8.8	64.5 ± 7.9	61.9 ± 9.1	0.073[a]
Sex, *n* (%)				
Male	113 (65.3)	52 (92.9)	61 (52.1)	<0.001[b]
Female	60 (34.7)	4 (7.1)	56 (47.9)	
Smoking status, *n* (%)				
Ever	117 (67.6)	53 (94.6)	64 (54.7)	<0.001[b]
Never	56 (32.4)	3 (5.4)	53 (45.3)	
Pathologic stages, *n* (%)				
Stage I	90 (52.0)	30 (53.6)	60 (51.3)	0.568[b]
Stage II	54 (31.2)	19 (33.9)	35 (29.9)	
Stage III	29 (16.8)	7 (12.5)	22 (18.8)	

[a] *t* test, SCC vs. AC.
[b] χ^2 test, SCC vs. AC.
AC, adenocarcinoma; SCC, squamous cell carcinoma; SD, standard deviation.

peripheral blood lymphocytes were obtained from 173 patients with NSCLC who underwent resection with curative intend at Kyungpook National University Hospital (Daegu, Korea) from January 2003 to July 2007. All patients included in this study were ethnic Koreans. Patients who underwent chemotherapy or radiotherapy before surgery were excluded to avoid the effects on DNA. The clinicopathologic characteristics of the patients are summarized in Table 1. Histologic type of the tumors was determined according to World Heath Organization criteria[16]: 56 tumors were squamous cell carcinomas (SCCs) (32.4%) and 117 were adenocarcinomas (ACs) (67.6%). There were 113 men (65.3%) and 60 women (34.7%), with age at diagnosis ranging from 40 to 82 years in men (median age, 64 years) and 35 to 79 years in women (median age, 62 years). Patients consisted of 56 never smokers (32.4%) and 117 smokers (67.6%). Of the 117 AC cases, 53 were never smokers (45.3%). Pathologic staging of lung cancers was determined according to the revised lung cancer staging system[17]: 90 (52.0%) had stage I disease, 54 (31.2%) stage II, and 29 (16.8%) stage III. All the tumor and macroscopically normal lung tissue samples were obtained at the time of surgery, rapidly frozen in liquid nitrogen and stored at −80°C. The slides of the tissue sections stained with hematoxylin and eosin were analyzed by a pathologist. Only specimens with greater than 80% tumor component were sent forward for DNA extraction and mutation analysis. All the macroscopically normal samples were confirmed as normal by hematoxylin and eosin staining.

Mutational Analysis of the EGFR Pathway Genes

In this study, the mutations in 11 EGFR signaling pathway genes—*EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *KRAS*, *NRAS*, *BRAF*, *PTEN*, *PIK3CA*, *LKB1*, and *AKT1*—were examined. We previously examined the same cohort for *TP53* (exons 2–11), *PTEN* (exons 1–9), and *LKB1* (exons 1–9) mutations.[14,15,18] Mutational analysis of *EGFR* (exons 18–21), *ERBB2* (exons 19 and 20), *ERBB3* (exons 2, 3, 7, and 8), *ERBB4* (exon 23), *KRAS* (exon 2), *NRAS* (exon 2), *BRAF* (exons 11 and 15), *PIK3CA* (exons 9 and 20), and *AKT1* (exon 3) was performed by polymerase chain reaction (PCR)-based direct sequencing. The primers and conditions for PCR reactions are shown in Supplementary Table 1 (available at: http://links.lww.com/JTO/A34). The PCR products were purified using a GENECLEAN Turbo kit (Q-Biogene, Carlsbad, CA). All sequence variants were confirmed by sequencing the products of independent PCR amplifications in both directions. Sequencing was done using an ABI Prism 3100 Genetic Analyzer (PE Biosystems, Foster City, CA). Nomenclature for the description of all sequence variations were according to the guidelines for mutation nomenclature by human genome variation society.[19]

Statistical Analysis

The association between mutations in EGFR signaling pathway genes and clinicopathological characteristics was analyzed using either a χ^2 test or Fisher's exact test. All statistical tests were two sided, and a p value less than 0.05 was considered statistically significant.

RESULTS

Of the 173 NSCLCs, a total of 65 mutations of EGFR pathway genes were detected in 63 tumors (36.4%). All the mutations found in the tumors were absent in DNA from the matched nonmalignant lung tissues and blood lymphocytes, indicating that these were somatic mutations. The frequency of mutations in each gene were 20.8% (36/173) for *EGFR*, 1.2% (2/173) for *ERBB2*, 6.4% (11/173) for *KRAS*, 1.2% (2/173) for *BRAF*, 2.9% (5/173) for *PIK3CA*, 4.6% (8/173) for *PTEN*, and 0.6% (1/173) for *LKB1*. There were no mutations observed in *ERBB3*, *ERBB4*, *NRAS*, or *AKT1*. There were two ACs with double mutations: one had an *EGFR* exon19 deletion mutation and a *PTEN* mutation (p.Tyr155His) and one had a *KRAS* mutation (p.Gly12Cys) and a *BRAF* mutation (p.Phe595Leu). Table 2 details mutations in each mutation-positive case.

EGFR mutations were more frequent in women than in men (35.0% versus 13.3%, $p < 0.001$) and in never smokers than in ever smokers (37.5% versus 12.8%, $p < 0.001$). In addition, the mutations were only detected in ACs (30.8%), and not in SCCs ($p < 0.001$) (Table 3). Of the 36 *EGFR* mutations, 26 (72.2%) were missense point mutations (p.Leu858Arg) in exon 21, seven (19.4%) were in-frame deletions in exon 19, and three (8.3%) were in-frame duplicating insertions in exon 20. There were two in-frame insertions in *ERBB2* in two ACs (1.7% in ACs). *KRAS* mutations were only detected in ACs (9.4%) and not in SCCs ($p = 0.02$). All the 11 *KRAS* mutations were missense point mutations at codon 12. There were two ACs carrying *BRAF* mutations in kinase domain: one (p.Gly469Val) in the glycine-rich GXGXXG motif within the phosphate binding loop of exon 11 and one (p.Phe595Leu) in the activation segment of exon 15. *PIK3CA* mutations were significantly more frequent in SCCs than in ACs (7.1% [4/56] versus 0.9% [1/117], $p = 0.04$). Of the 5 *PIK3CA* gene mutations, three were missense point mutations in the helical domain (p.Glu542Lys, p.Glu545Ala,