Epidermal Growth Factor Receptor Mutations in 510 Finnish Non–Small-Cell Lung Cancer Patients

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Introduction: Among the driver gene mutations in non–small-cell lung cancer, mutations in epidermal growth factor receptor (EGFR) are the most important because of their predictive role in selecting patients eligible for targeted therapy. Our aim was to study EGFR mutations in a Finnish non–small-cell lung cancer cohort of 528 patients.

Methods: Mutation testing was conducted on DNA extracted from paraffin-embedded, formalin-fixed tumor material using the following real-time polymerase chain reaction-based kits: Therascreen EGFR PCR Kit and cobas EGFR Mutation Test.

Results: EGFR mutation frequency was 11.4% and all positive cases were adenocarcinomas, of which a majority had an acinar predominant pattern. Mutations were seen significantly more often in females and never-smokers than in males and smokers. The most frequent mutations were L858R in exon 21 and deletions in exon 19. Overall survival of the patients, not treated with EGFR inhibitor, did not differ between EGFR mutation-positive and EGFR mutation-negative patients.

Conclusion: EGFR mutation profile in this Finnish non–small-cell lung cancer cohort resembles in many respect with that of other Western European cohorts, even though the overall frequency of mutations is slightly higher. We show the occurrence of EGFR mutations in patients with occupational asbestos exposure and also in those diagnosed with chronic obstructive pulmonary disease who have not been often investigated before.

Key Words: EGFR, Mutations, Frequency, Lung adenocarcinoma, Non–small-cell lung cancer.

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Among the driver gene mutations in non–small-cell lung cancer (NSCLC), mutations in epidermal growth factor receptor (EGFR) are the most important because of their predictive role in selecting patients eligible for targeted therapy.[1] EGFR mutation frequency and histologic subtype distribution in Finnish NSCLC patients have not been studied earlier. Because of the special heritage history of the Finnish population and the influence of ethnic background on the incidence of EGFR mutations, it is reasonable to study the Finnish population separately. The aim of our study was to examine EGFR mutation frequency in a cohort of 528 consecutive Finnish Caucasian NSCLC patients by using real-time polymerase chain reaction performed on DNA extracted from formalin-fixed, paraffin-embedded tumor material.

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MATERIALS AND METHODS

Patients

In total, we collected 613 formalin-fixed, paraffin-embedded specimens, including 610 tumor and 3 pleural effusion specimens, of NSCLC patients treated at the Hospital District of Helsinki and Uusimaa (HUS), Finland, during 2006 to 2012 (primary diagnosis for three patients in 2004). Tumor and pleural effusion specimens were collected upon diagnosis or the surgical operation. In total, 528 specimens were eligible to be tested for EGFR mutation status. Tumor cell content of the specimens ranged from 2 to 98%; in 87% of the samples, tumor cell content was at least 20%. Of the patients with a test obtained, 53% were male, 77% had been diagnosed with adenocarcinoma (ADC), 12% with squamous cell carcinoma, 8% with large cell carcinoma, and 3% had other subtype or not otherwise specified type of NSCLC. The other subtype/not otherwise specified included 10 patients diagnosed with adenosquamous carcinoma, four patients with not otherwise specified NSCLC, and three patients with sarcomatoid carcinoma. Histologic diagnosis was based on pathologist's evaluation according to the World Health Organization criteria. EGFR-positive cases were subtyped according to the updated International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification.[2] Clinicopathologic characteristics of the patients are presented in Table 1.

DNA Extraction and Mutation Detection

DNA from 496 formalin-fixed, paraffin-embedded tumor tissue specimens of NSCLC patients was extracted using

QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) as described earlier.[3] Tumor DNA samples of 528 NSCLC patients were tested for EGFR mutations using the Therascreen EGFR PCR Kit (Qiagen, Manchester, United Kingdom) according to the manufacturer's protocol, on the ABI7500 platform or the cobas EGFR Mutation Test (Roche Molecular Systems, South Branchburg, NJ; 28 patients) according to the manufacturer's protocol, on the cobas z480 platform.

RESULTS

The total frequency of EGFR mutations was 11.4% (58 of 510). The mean age of patients with EGFR mutation was higher (69.2 ± 6.9 years) compared with patients with wild-type EGFR (65.2 ± 8.8 years, $p = 0.001$). EGFR mutations occurred more often in women (16.6%, 40 of 241) than in men (6.7%, 18 of 269, $p < 0.001$) and in never-smokers (32.8%, 22 of 67) than in ever-smokers (8.2%, 36 of 438, $p < 0.001$). A group of ever-smokers was divided into the following subgroups: light ex-smokers (smoking <20 years, cessation >10 years ago), medium ex-smokers (smoking >20 years, ceased), and current smokers (current smokers, smoking >20 years). Of the EGFR-mutated patients, 38% (22 of 58) were never-smokers, 19% (11 of 58) light ex-smokers, 24% (14 of 58) medium ex-smokers, and 19% (11 of 58) current smokers.

All mutations occurred in patients diagnosed with ADC. Vast majorities, 98%, of EGFR mutation-positive ADCs were invasive ADCs (53 of 54 with sufficient material for reliable reclassification) and one (2%) was diagnosed with nonmucinous minimally invasive ADC. Of the 54 EGFR-positive ADCs, 40 (74%) were acinar predominant, of which 18 were of the mixed acinar type: 10 with papillary (one of these had also micropapillary pattern and one all four patterns), four with lepidic, three with solid pattern, and one with nonmucinous minimally invasive ADC. The other EGFR-positive subtypes of invasive ADCs were lepidic (9%, five of 54), solid (7%, four of 54), micropapillary (4%, two of 54), and papillary predominant (4%, two of 54).

The most frequent mutations were amino acid change L858R in exon 21 and deletions in exon 19, representing 41.4% (24 of 58) and 36.2% (21 of 58) of all mutations, respectively. Other mutations consisted of G719X (8.6%, five of 58), L861Q (3.4%, two of 58), S768I (1.7%, one of 58), and insertions in exon 20 (1.7%, one of 58), together representing 15.5% of all EGFR mutations. Also four double EGFR mutants (6.9%) were detected: two patients with

TABLE 1. Clinicopathologic Characteristics of the Patients Studied

	Total, N (%)*	Tested, N (%)*	Result obtained, N (%)*	EGFR wt, N (%)†	EGFR+, N (%)†	*p* Value
Total	613	528	510	452	58	
Histology						
ADC	460 (75.0)	411 (77.8)	398 (78.0)	340 (85.4)	58 (14.6)	< 0.001
SCC	77 (12.6)	60 (11.4)	60 (11.8)	60 (100)	0	
LCC	43 (7.0)	40 (7.6)	38 (7.5)	38 (100)	0	
NSCLC NOS	33 (5.4)	17 (3.2)	14 (2.7)	14 (100)	0	
Gender						
Male	339 (55.3)	279 (52.8)	269 (52.7)	251 (93.3)	18 (6.7)	<0.001
Female	274 (44.7)	249 (47.2)	241 (47.3)	201 (83.4)	40 (16.6)	
Age, years						
Mean	65.9	65.7	65.7	65.2	69.2	
Range	26–90	26–90	26–90	26–90	55–85	
Smoking						
Never	79 (12.9)	69 (13.1)	67 (13.1)	45 (67.2)	22 (32.8)	<0.001
Light	53 (86.5)	51 (9.7)	51 (10.0)	40 (78.4)	11 (21.6)	
Medium	211 (34.4)	178 (33.7)	173 (33.9)	159 (91.9)	14 (8.1)	
Current	264 (43.1)	225 (42.6)	214 (42.0)	203 (94.9)	11 (5.1)	
Data missing	6	5	5	5	0	
Asbestos						
Exposed	57 (9.3)	48 (9.1)	46 (9.0)	41 (89.1)	5 (10.9)	0.498
Non-exposed	261 (42.6)	226 (42.8)	219 (43.0)	198 (90.4)	21 (9.6)	
No sure information	295 (48.1)	254 (48.1)	245 (48.0)	213 (86.9)	32 (13.1)	
COPD						
Yes	125 (20.4)	113 (21.4)	111 (21.8)	104 (93.7)	7 (6.3)	0.057
No	488 (79.6)	415 (78.6)	399 (78.2)	348 (87.2)	51 (12.8)	

*Proportions calculated from all patients in the group (column) in question.
†Proportions calculated from a total number of variable group (row) in question.
ADC, adenocarcinoma; COPD, chronic obstructive pulmonary disease; LCC, large cell carcinoma; NSCLC NOS, non-small-cell lung cancer.

