EGFR Gene Alterations in a Norwegian Cohort of Lung Cancer Patients Selected for Surgery

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Introduction: Lung cancer is the leading cause of cancer-related deaths worldwide. New therapies targeting the epidermal growth factor receptor (EGFR) tyrosine kinase are promising and show high response rates in the subset of patients with activating mutations in EGFR. The frequency of these mutations is largely unknown in unselected Caucasian patients.

Methods: Mutation analysis of EGFR exons 18–21 was performed on 240 lung cancer samples using the TheraScreen EGFR mutation kit and denaturing high-performance liquid chromatography in addition to sequencing.

Results: In a cohort of 240 Norwegian lung cancer patients selected for surgery, we identified 18 tumors with EGFR-activating mutations (7.5%, 14 women and 4 men), of which 14 were adenocarcinomas, 2 squamous cell carcinomas, and 2 bronchoalveolar carcinomas. Five of the mutations were found in patients with more than 20 pack-years of smoking history.

Conclusion: The frequency of EGFR mutations is lower in our cohort than among Asian lung cancer patients and present in both men and women and smokers and never-smokers. However, the frequency is significantly higher among women and never-smokers and among patients with adenocarcinomas.

Key Words: Lung cancer, EGFR mutations, Frequency, Caucasian.

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Lung cancer accounts for approximately one-third of all cancer deaths worldwide. Non-small cell lung carcinoma (NSCLC) can be categorized into several subtypes determined by specific molecular events, which may be mirrored by the etiology. Analyses show that NSCLC among smokers and never-smokers have different molecular characteristics.

One pivotal molecular event in subsets of NSCLC is dysregulation of epidermal growth factor receptor (EGFR). During the past years, several agents that target the EGFR have been developed. Early studies identified the presence of activating mutations in the kinase domain of the EGFR gene as a marker of response,[1–3] and the predictive value has recently also been shown in clinical randomized studies.[4] EGFR mutations have been found more frequently among Asian, nonsmoking, female patients and are also present in Caucasians.[5] In a Spanish lung cancer cohort, approximately 16% of patients were found to have EGFR mutations, but most of these patients were never-smokers.[5,6] Little is known about the exact frequency of EGFR mutations in Northern European patients with lung cancer. In this study, we report the frequency of EGFR mutations in exons 18–21 by both quantitative-polymerase chain reaction (PCR) and sequencing in a Norwegian lung cancer patient cohort.

MATERIALS AND METHODS

Material

Tumor tissue was obtained from 240 operable lung cancer patients admitted to Oslo University Hospital-Rikshospitalet during the period 2006–2010. Tissue was taken from the excised tumors, snap frozen in liquid nitrogen in the operation room, and stored at −80°C until DNA isolation. The tumor cell content in the specimens was found to be more than 70% in most samples. DNA was isolated from the biopsies using chloroform/phenol extraction followed by ethanol precipitation using the Applied Biosystems (Foster City, CA) 340A Nucleic Acid Extractor according to standard protocol.

The project was approved by the institutional review board and regional ethics committee, and written consent was obtained from each participant.

Mutation Analyses

Mutation analysis of EGFR exons 18–21 was performed on all samples using the TheraScreen EGFR mutation kit (DxS, Manchester, UK). The assay is designed to detect 28 specific mutations in the EGFR gene by real-time PCR.

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It enables the detection of 19 distinguished deletions in exon 19, L858R, L861Q, G719X, S768I, and three insertions in exon 20. Assays were carried out according to the manufacturer's protocol and using the Roche LightCycler 480 real-time PCR system. Data analyses were performed using the LightCycler Adapt software (LightCycler 480 Software, v1.5).

We additionally analyzed 107 of the samples by denaturing high-performance liquid chromatography (dHPLC) followed by sequencing. After amplification, DNA heteroduplexes were detected on a WAVE 3500HT instrument (Transgenomic, Glasgow, UK) according to manufacturer's standard procedure. Samples displaying aberrant dHPLC chromatograms were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA). Capillary electrophoresis was performed on the 3130×l Genetic Analyzer (Applied Biosystems). Mutation Surveyor (v3.30) and Sequence Scanner (v1.0, Applied Biosystems) were used for sequence analysis.

Immunohistochemistry

Paraffin-embedded tissue was pretreated with Proteinase-K (DAKO) for antigene retrival. The following staining procedure was performed according to the standardized protocol EnVision FLEX+, Mouse, High pH (Dako Autostainer/Autostainer Plus).

Statistical Analyses

Statistical analyses were performed using SPSS 16. Relapse-free survival was defined as time between surgery and relapse or death due to lung cancer. The Kaplan-Meier method[7] and the log-rank test were used to estimate and compare survival rates. Statistical significance was defined as $p < 0.05$.

RESULTS

Patient Characteristics

Two hundred forty patients diagnosed with operable lung cancer were included in this study. This is 53% of the total number of lung cancer patients operated at our hospital during this period of time and selected based on practicalities (study nurse resources). The characteristics according to gender, stage (TNM-7), histology, and smoking status is presented in Table 1. Median age was 64 and 66 years for female and male patients, respectively. One hundred forty-one patients (54%) were diagnosed with adenocarcinoma and 63 (26%) with squamous cell carcinoma (Table 1). Fifteen patients (6.2%) were never-smokers while 83 (34%) were previous smokers, defined as having stopped smoking at least 1 year before surgery.

Adjuvant chemotherapy was offered patients based on their TNM stage and age, according to national guidelines. There were no significant difference in frequency of patients receiving adjuvant chemotherapy between patients with mutation (28%) and wild-type EGFR gene (26%).

TABLE 1. Characteristics of the Patients in This Cohort

	Total	Mutated	*p*
Gender			0.008
Female	116	14	
Male	124	4	
Total	240	18	
T stage			NS
T1	81	6	
T2	129	11	
T3	17	0	
T4	13	1	
N stage			NS
N0	175	11	
N1	44	5	
N2	21	2	
Histology			0.017
Adenocarcinoma	141	16	
Squamous cell carcinoma	63	2	
Large cell carcinoma	23	0	
Carcinoid	11	0	
Adenosquamous	1	0	
SCLC	1	0	
p Stage			NS
I	141	10	
II	44	5	
III	34	3	
IV	3	0	
Smoking			0.000
Current smoker	142	2	
Former smoker	83	8	
Never smoker	15	8	

SCLC, small cell lung cancer; NS, not significant.

Mutations

We identified EGFR mutations in a total of 18 of the 240 tumors (7.5%) (Table 2). Sixteen of the mutations were detected among the 141 adenocarcinomas (11%) and 2 among the 63 squamous cell carcinomas (3%). The distribution of tumor stage was similar in the mutated and the wild-type tumors. Median age in mutation-positive patients was 66.5 years in females and 68.5 years in men.

We found mutations in eight of the never-smoking patients (53%), eight of previous smokers (10%), and two of the current smoking patients (1.4%) (Table 1). Fourteen of the mutations (78%) were identified in female patients. Among patients with wild-type EGFR gene, the median tobacco consumption was 29 pack-years, while median pack-years among mutated were 1.3. Five mutation-positive patients had smoked more than 20 pack-years. Females had significantly higher mutation frequency regardless of smoking history ($p < 0.0001$). We identified one L861Q mutation and one G719X mutation, both in heavy smokers, and one of the L858R mutations was also detected in a heavy smoker.

One hundred seven of the 240 samples were also analyzed by dHPLC combined with sequencing. dHPLC