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The Role of Mutation Status of the Epidermal Growth Factor Receptor Gene In Advanced Non–Small Cell Lung Cancer

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Key words: *non–small cell lung cancer; EGFR mutations; tyrosine kinase inhibitor.*

Summary. *Objective. The aim of this study was to examine the prevalence of epidermal growth factor receptor (EGFR) gene mutations among patients with advanced nonsquamous non–small cell lung cancer (NSCLC) treated in our institution and to evaluate the associations between EGFR mutations and clinicopathological characteristics.*

Materials and Methods. A total of 103 patients with NSCLC were examined from April 2010 to September 2011. The patients were screened for EGFR mutations in exons 19 and 21 using sequence analysis.

Results. EGFR mutations were detected in 10 patients (9.71%): 23.1% of women and 5.2% of men (P<0.05), 31.8% of never-smokers and 4.7% of smokers (P<0.05), and 12.3% of patients with adenocarcinomas and 6.25% of patients with large cell carcinomas (P>0.05). Eight mutations (80.0%) were found in exon 21: 7 patients had the L858R mutation and 1 patient had the L861G mutation. Two mutations (20.0%) were found in exon 19: 1 patient had the L747-A748 deletion and 1 patient had the L747-A750insE deletion. The overall response rate was significantly greater in the EGFR mutation-positive group than in the EGFR mutation-negative or control groups (P<0.05). The median progression-free survival in the EGFR mutation-negative group and the control group that received systemic standard chemotherapy was 5.6 months (95% CI, 4.3 to 7.0) and 5.3 months (95% CI, 4.9 to 5.7), respectively, but it was not achieved in the EGFR mutation-positive group that received EGFR tyrosine kinase inhibitors (P<0.05).

Conclusions. The frequency of EGFR mutations in our patients with nonsquamous NSCLC was found to be similar to that reported in Europe. EGFR mutations were more frequent in women and never-smokers.

Introduction

Lung cancer is the major cause of cancer-related mortality in men and women worldwide (1). Nonsquamous non–small cell lung cancer (NSCLC) accounts for the majority of cases, and patients with advanced NSCLC are at higher risk of poor prognosis. To date, platinum-based chemotherapy was the standard treatment method for advanced or recurrent NSCLC. The outcome of this treatment was limited: the mean response rate was around 30% (2), and the median survival ranged from 8 to 10 months (3). In recent years, the advancement of biomarker-driven personalized therapy has changed an approach to the treatment of many cancers, including NSCLC. Two target-directed therapies, such as epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) and antivascular endothelial growth factor (anti-VEGF) monoclonal antibody, have been approved in the treatment of NSCLC (4–7). In addition, other therapies targeting hepatocyte growth factor receptor (MET), echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase (EML4-ALK), receptor tyrosine protein kinase erbB-2 (HER2), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform (PIC3CA), serine/threonine protein kinase B-raf (BRAF), insulin-like growth factor 1 receptor (IGF-1R), and others are in the clinical testing (5).

The *EGFR* gene is located on the short arm of chromosome 7 (*EGFR* is a P11.2) and encodes a 170-kDa type I transmembrane growth factor receptor. *EGFR* belongs to the HER/erbB family of receptor tyrosine kinase. This receptor has an ex-

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tracellular cysteine-rich ligand-binding domain and an intracellular domain possessing intrinsic tyrosine kinase activity. Intracellular signaling is mediated mainly through the RAS-RAF-MEK-MAPK pathway, the PI3K-PTEN-AKT pathway, and the signal transducer and activator of transcription (STAT) pathway (8). These EGFR signaling pathways are important in tumor cell growth, local invasion, angiogenesis, protein translation, autophagy, and cell metabolism (9). The potential relevance of *EGFR* gene mutations to NSCLC treatment has been identified in 2004 (10–12). These mutations cause a constitutive activation of the tyrosine kinase domain of the *EGFR* gene and are found in approximately 10%–20% of white patients and more than 30% of East Asian patients with NSCLC and are strongly associated with some clinicopathological characteristics of patients (13–15). Mutations of the *EGFR* gene have been proved to predict the activity of EGFR-TKIs (16). Two EGFR-TKIs, erlotinib and gefitinib, are approved for the treatment of NSCLC. These low-molecular-weight agents selectively inhibit the activity of the intracellular *EGFR* tyrosine kinase domain.

However, the frequency of *EGFR*-activating mutations and its predictive role in patients with NSCLC has not been studied in Lithuania yet. The aim of the study was to examine the prevalence of *EGFR*-activating mutations among patients with histologically confirmed nonsquamous NSCLC treated in our institution and to evaluate the associations between *EGFR* mutations and clinicopathologic characteristics.

Material and Methods

The patients with NSCLC enrolled into the study from April 2010 to September 2011 at the Hospital of Lithuanian University of Health Sciences were examined. The mutations of the *EGFR* gene in exons 19 and 21 of 103 patients with diagnosed locally advanced or metastatic nonsquamous NSCLC not suitable for radical treatment were studied. Fifty-four patients with advanced NSCLC, who received systemic standard chemotherapy and who were not screened for *EGFR* mutations, were included in our study as the control group.

The clinical stage, tumor type, and performance status of lung cancer were recorded at the time of diagnosis before receiving anticancer therapy according to the Eastern Cooperative Oncology Group (ECOG) (17). NSCLC stage was determined according to the *TNM Classification of Malignant Tumours*, the Seventh Edition (18).

Mutation Analysis. Formalin-fixed paraffin-embedded tissue samples were obtained by tumor biopsy before any treatment. Slides were reviewed by a pathologist to assure greater than 50% tumor content as suitability for DNA extraction. QIAamp® DNA FFPE Tissue Kits for extraction of human DNA from formalin-fixed paraffin-embedded tumor samples were used (QIAGEN® kit, Germany). Single-stranded DNA was prepared, and the corresponding sequencing primers annealed to DNA. Two separate PCR amplifications of regions containing codons 858–861 (exon 21) or deletions and complex mutations in exon 19 using the *therascreen* EGFR Pyro primers were employed. After PCR using the primers targeting exons 19 and 21, the amplicons were immobilized on Streptavidin Sepharose® High Performance beads. The samples were then analyzed on the PyroMark Q24 system (Germany) using a run setup file and a run file. Unmethylated control DNA was included in the run as a positive control for PCR and sequencing reactions. In addition, a negative control (without template DNA) was included in every PCR setup for at least one assay.

The study subjects were divided into 3 categories according to their smoking status: never smokers (<100 lifetime cigarettes), former smokers (⩾1 year since cessation), and current smokers (still smoking, or <1 year since cessation). Smoking history was calculated in pack-years as the product of tobacco use (in years) and the average number of cigarettes smoked per day/20 (years × cigarettes per day/20).

Tumor response to treatment was evaluated using the Response Evaluation Criteria In Solid Tumors (RECIST) guidelines (19). The duration of progression-free survival was calculated from the date of treatment initiation to the date of disease progression or death.

Kaunas Regional Ethics Committee for Biomedical Research approved the study, and written informed consent was received from all the participants.

Statistical Analysis. Statistical analysis was performed using the statistical SPSS 18.0 software package for Windows. Data are presented as mean (standard deviation). The associations between the *EGFR* status and clinicopathologic characteristics were analyzed using the chi-square (χ^2) test or the Fisher exact test. Differences among all study groups (more than two groups) were evaluated using the Kruskal-Wallis test. Differences between two groups were evaluated using the Mann-Whitney *U* test. Progression-free survival was analyzed by the Kaplan-Meier method and compared with the log-rank test. Statistical significance was assumed at $P<0.05$.

Results

The characteristics of the study population are summarized in Table 1.

Table 1. Clinicopathological Features of the Study Population

Variable	Group		
	EGFR Mutation Positive n=10	*EGFR* Mutation Negative n=93	Control n=54
Gender			
Male	4 (40.0)*	73 (78.5)**	50 (92.6)
Female	6 (60.0)*	20 (21.5)	4 (7.4)
Age, mean (SD), years	61.1 (10.9)	62.7 (11.2)	66.4 (10.5)
Smoking history			
Never smoker	7 (70.0)*	15 (16.1)	6 (11.1)
Former smoker	0	17 (18.3)	8 (14.8)
Current smoker	3 (30.0)*	61 (65.6)	40 (74.1)
Disease stage			
Stage IIIB	1 (10.0)	20 (21.5)	15 (27.8)
Stage IV	9 (90.0)	73 (78.5)	39 (72.2)
Histologic type			
Adenocarcinoma	8 (80)**	57 (61.3)**	11 (20.4)
Large-cell carcinoma	2 (20)	30 (32.2)**	4 (7.4)
NSCLC-NOS	0	6 (6.5)	8 (14.8)
Squamous-cell carcinoma	0	0	31 (57.4)

Values are number (percentage) unless otherwise indicated. EGFR, epidermal growth factor receptor; NSCLC, non–small cell lung cancer; NSCLC-NOS, non–small cell lung cancer not otherwise specified.
*$P<0.05$ compared with the *EGFR* mutation negative and control groups.
**$P<0.05$ compared with the control group.

Table 2. Clinicopathological Features of Patients With *EGFR* Mutations

Gender	Age at Diagnosis, Years	Histology	Smoking History, (Pack-Years)	Exon	Alteration	Amino Acid
Female	60	Adenocarcinoma	Never smoker	21	Substitution	L858R
Female	76	Adenocarcinoma	Never smoker	21	Substitution	L858R
Female	70	Adenocarcinoma	Never smoker	21	Substitution	L861G
Female	67	Adenocarcinoma	Never smoker	19	Deletion	L747-R748
Female	64	Large cell carcinoma	Never smoker	21	Substitution	L858R
Female	41	Large cell carcinoma	Current smoker (4 pack-years)	21	Substitution	L858R
Male	72	Adenocarcinoma	Never smoker	21	Substitution	L858R
Male	61	Adenocarcinoma	Never smoker	21	Substitution	L858R
Male	52	Adenocarcinoma	Current smoker (30 pack-years)	21	Substitution	L858R
Male	51	Adenocarcinoma	Current smoker (35 pack-years)	19	Deletion	L747-A750insE

EGFR, epidermal growth factor receptor.

Mutations in the *EGFR* gene were detected in 10 (9.71%) of the 103 patients. Clinicopathological features of the studied patients with the mutations of the *EGFR* gene are described in Table 2.

No significant differences in the frequency of *EGFR* mutations was identified comparing adenocarcinomas and large-cell carcinomas: *EGFR* mutations were present in 8 (12.3%) of the 65 adenocarcinomas and in 2 (6.25%) of the 32 large-cell carcinomas ($P>0.05$), but not found in NSCLC not otherwise specified (NOS). *EGFR* mutations were detected more frequently in women than men (23.1%, 6/26 vs. 5.2%, 4/77; $P<0.05$) and in patients who had never smoked than current smokers (31.8%, 7/22 vs. 4.7%, 3/64; $P<0.05$). There were no significant differences in smoking intensity comparing the *EGFR* mutation-positive, *EGFR* mutation-negative, and control groups (23.0 [SD, 16.6], 33.8 [SD, 13.4], and 39.0 [SD, 12.5] pack-years, respectively; $P>0.05$).

In our study, *EGFR* mutations were found more frequently in exon 21 than exon 19 ($P<0.05$). Eight mutations (80.0%) were found in exon 21: 7 patients had the L858R mutation and 1 patient had the L861G mutation. Two mutations (20.0%) were found in exon 19: 1 patient had the L747-A748 deletion and 1 patient had the L747-A750insE deletion (Table 2).

Table 3. Response Rate to Systemic Treatment by Study Groups

Response	Group		
	EGFR Mutation Positive (n=7)	*EGFR* Mutation Negative (n=73)	Control (n=54)
Overall response	6 (85.7)*	24 (32.9)	17 (31.5)
Stable disease	0	29 (39.7)	20 (37.0)
Progressive disease	1 (14.3)	20 (27.4)	17 (31.5)

Values are number (percentage). EGFR, epidermal growth factor receptor.
*$P<0.05$ compared with the *EGFR* mutation negative and control groups.

At the time of analysis, the median follow-up of study patients was 5.3 months (range, 0.1 to 17.4). Seven patients harboring *EGFR* mutations were treated with EGFR-TKIs (gefitinib or erlotinib). Three patients (42.9%) developed grade 2 skin side effects, and 1 patient (14.3%) had grade 1 diarrhea. The patients without *EGFR* mutations as well as the control group were treated with standard chemotherapy. The overall response rate according the RECIST was significantly greater in the *EGFR* mutation-positive group than in the *EGFR* mutation-negative or control groups (Table 3). The median progression-free survival in the *EGFR* mutation-negative group and control patients who received systemic standard chemotherapy was 5.6 months (95% CI, 4.3 to 7.0) and 5.3 months (95% CI, 4.9 to 5.7), respectively, but it was not achieved in the *EGFR* gene mutation-positive group ($P<0.05$) (Fig.). There were no significant differences in the progression-free survival by gender, smoking history, stage, and histologic type.

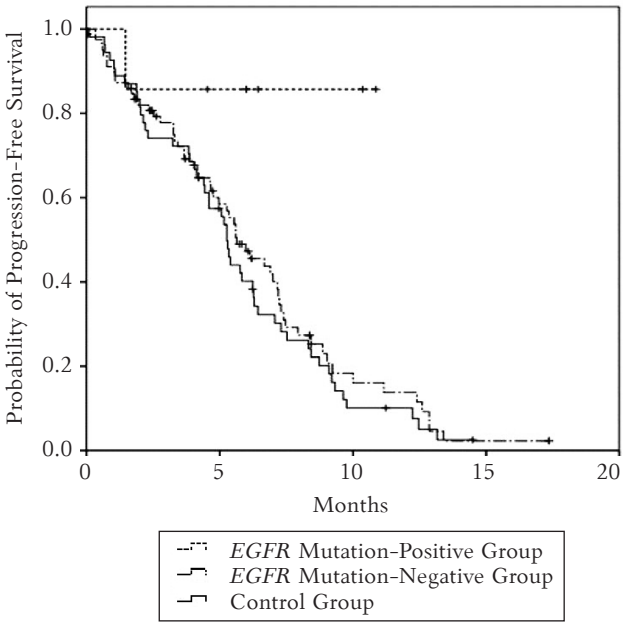

Fig. Kaplan-Meier curve for progression-free survival of patients by study groups

*$P<0.05$ compared with the epidermal growth factor receptor (*EGFR*) mutation negative group and control group (log-rank test).

Discussion

The identification of predictive markers among patients with locally advanced or metastatic NSCLC is important for the selection of treatment with EGFR-TKIs. Multiple clinical and pathological factors, such as female gender, no smoking history, Asian ethnicity, and adenocarcinoma histology, are associated with response to the treatment with EGFR-TKIs. However, *EGFR* gene mutations are the most important predictive markers of sensitivity to the treatment with EGFR-TKIs.

It is a first single-institution experience of *EGFR* mutation status in Lithuanian patients with NSCLC. Our results showed the rates of *EGFR* mutations to be 9.71% in the case of NSCLC. These results are comparable with those of other studies in Europe, where the frequency of *EGFR* mutations was shown to range from 10% to 16.6% in the Caucasian population (15, 20, 21). Meanwhile, among East Asian patients with NSCLC, the frequency of *EGFR* mutations was reported to be from 40% to 64% (6, 7, 13, 14).

The frequency of *EGFR* mutations varies not only with the ethnicity, but also with gender, smoking status, and histologic type of NSCLC. Literature data have shown that mutations are more common in women than men (42% vs. 14%), in patients who have never smoked than those who have smoked (51% vs. 10%), and in patients with adenocarcinomas than those with other histologic types (40% vs. 3%) (9–13, 22–24). In our study, *EGFR* mutations were also significantly more frequent in women and nonsmokers. These results are in line with those recently reported (10–12). Interestingly, the higher mutation rates among patients who have never smoked and patients with adenocarcinoma are consistent in both East Asian and white populations (24). The presence of *EGFR* mutations in smokers and men proves once again that demographic and clinical features are not sufficiently predictive NSCLC markers of sensitivity to the treatment with EGFR-TKIs.

In our study, the frequency of *EGFR* mutations in adenocarcinomas was 12.5%; in large cell carcinomas, 6.1%; and in NSCLC NOS histology, 0.0%. However, in contrast to other investigations, no significant association between *EGFR* mutation and histological type was found, and this may be explained by the small number of patients with these mutations. Rossel et al. reported that *EGFR* mutations were present in 16.6% of cases with adenocarcinomas and 11.5% of cases with large-cell carcinomas (15). Marchetti et al. studied 375 patients with lung adenocarcinomas, and the frequency of *EGFR* mutations was found to be was only 10%. There were no *EGFR* mutations in 31 patients with large-cell carcinoma (20). Among adenocarcinomas, *EGFR* mutations were found to be more prevalent in cases of bronchioalveolar carcinoma (BAC) of mixed subtype with acinar and BAC components (25). The modified 2004 World Health Organization classification of lung adenocarcinomas, which included the adenocarcinomas of mixed subtypes, corresponds to the previous classification of adenocarcinomas with BAC features (26). *EGFR* mutations are very rare in squamous cell carcinoma and are detected only if some adenocarcinoma component is presented (13, 20, 27). There are no sufficient data about the frequency of *EGFR* mutations in this subset of lung carcinomas in the Caucasian population. Tochigi et al. studied 23 patients with lung adenosquamous carcinomas, and *EGFR* mutations were found in 3 cases (13%) (28). These data suggest that not only patients with adenocarcinomas, but also with large cell carcinomas and adenosquamous carcinomas (but not with squamous-cell carcinomas), should be screened for *EGFR* mutations. More precise classification of NSCLC NOS (favor adenocarcinoma or squamous cell carcinoma) according to the new classification of adenocarcinomas is important (29).

Small deletions in exon 19 (35%–45% of all *EGFR* mutations) that eliminate amino acids 747–750 (Leu-Arg-Gliu-Ala) and point mutations in exon 21 that result in the amino acid substitution L858R (35%–48% of all *EGFR* mutations) are the most common mutations of the *EGFR* gene (10–13). In our study as compared with the literature data, the lower frequency of deletions in exon 19 was found. These results may be influenced by a small sample size or differences in ethnicity.

In 2004, several groups of investigators initially reported about the presence of activating mutations of the *EGFR* gene (10–12). Patients with lung cancers that harbor base-pair deletions in exon 19 and the L858R mutation in exon 21 respond very well to treatment with EGFR-TKIs, such as gefitinib and erlotinib, as compared with those without *EGFR* mutations treated with standard chemotherapy. These mutations have been associated with improved outcomes after treatment with EGFR-TKIs (erlotinib or gefitinb) (9–12, 23). Recent studies suggest that patients with NSCLC and *EGFR* deletion in exon 19 have a longer survival following treatment with EGFR-TKIs compared with those harboring L858R mutations (30–32). The Iressa Pan-Asia Study (IPASS) was the first open-label, randomized, phase 3 trial comparing gefitinib versus standard chemotherapy as a first-line treatment of patients with locally advanced or metastatic NSCLC (6). This study was conducted in the Asian population and reported the response rates of 71.2% to gefitinib among patients with *EGFR* mutations, with a median progression-free survival of 9.6 months. Two randomized phase 3 trials comparing erlotinib versus chemotherapy as a first-line treatment in NSCLC with *EGFR*-activating mutations were the European Randomized Trial of Tarceva vs. Chemotherapy (EURTAC) and the OPTIMAL trial. The EURTAC trial was conducted in the Caucasian population from Spain, Italy, and France, and the OPTIMAL trial was conducted in the Asian population from China. In the EURTAC trial, the researchers screened 1275 patients over a 5-year period to compose the study population of 174 patients who were randomly assigned to receive erlotinib or platinum-based chemotherapy. This study reported a response rate of 54.3% to erlotinib among patients with *EGFR* mutations with a median progression-free survival of 9.7 months (21). Other randomized, phase 3 trial OPTIMAL (7) showed similar results to the EURTAC and IPASS trials. Interestingly, Rossel et al. (15) have reported that the response to EGFR-TKIs is the same whether they are administered as the first-line therapy or the second-line therapy for patients with *EGFR* mutations. Our results are similar with reported previously; the response rate and the median progression-free survival in patients with *EGFR* mutations treated with EGFR-TKIs were significantly improved compared with the patients without *EGFR* mutations and the control group.

The most common adverse events in patients receiving EGFR-TKIs were cutaneous toxicity (skin rash, dry skin), diarrhea, and liver dysfunction. The majority of these events were mild or moderate in intensity, and severe adverse events were infrequent (6, 7). On the contrary, in the patients treated with EGFR-TKIs, a significantly lower incidence of emesis, fatigue, hematological toxicity, and hair loss was documented as compared with the patients treated with chemotherapy. In our study, EGFR-TKI treatment-related toxicity was generally mild. Only 3 patients developed skin grade 2 side effects, and 1 patient had grade 1 diarrhea.

Conclusions

The frequency of *EGFR* mutations in our patients with nonsquamous NSCLC is similar to that reported in Europe. *EGFR* mutations were more frequent in women and never-smokers. *EGFR* mutations are still the most effective molecular marker of sensitivity to EGFR-TKI treatment in patients with advanced NSCLC.

Statement of Conflict of Interest

The authors state no conflict of interest.

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