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ORIGINAL ARTICLE

The Frequency of EGFR Mutation in Lung Adenocarcinoma and the Efficacy of Tyrosine Kinase Inhibitor Therapy in a Hungarian Cohort of Patients

Veronika Sárosi[1] · Zoltán Balikó[1] · Gábor Smuk[2] · Terézia László[2] · Mariann Szabó[1] · István Ruzsics[1] · Emese Mezősi[1]

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Abstract In the last decades new therapeutic drugs have been developed for the treatment of non-small cell lung cancer (NSCLC) patients. Tyrosine kinase inhibitors (TKIs) significantly increase the progression free survival (PFS) of patients with NSCLC carrying epidermal growth factor receptor (EGFR) mutations. This type of lung cancer occurs mainly among non-smoking women and Asian origin. However, the new ESMO guideline recommends EGFR mutation analysis in every patient with NSCLC, because in patients with activating EGFR mutation, TKIs should be considered as first line therapy. In our recent work, we analyzed data of patients with EGFR-mutant adenocarcinoma from January 2009. The number of patients investigated was 446, among them 44 cases were positive for EGFR mutation. The ratio of positive cases was 9.86 % that is lower than the average mutation rate in Europe and much lower than that found in Asia. The exon 19 deletion was detected in 61.4 % of the patients, while L858R point mutation in exon 21 was observed in 34.1 % of them. In one subject, both exon 19 and 21 mutations were present simultaneously. A rare mutation located in exon 21 was found in another patient. TKI therapy was conducted in 38 patients. The disease control rate by TKI therapy was 85.7 %; primary resistance was documented in five subjects. Non-smoking patients with EGFR mutant adenocarcinoma had the highest benefit from TKI treatment. Our data support the recommendation that EGFR mutation status should be defined in all cases of locally advanced or metastatic lung adenocarcinoma.

Keywords NSCLC · Adenocarcinoma · EGFR mutation · TKI

✉ Emese Mezősi
mezosi.emese@pte.hu

[1] Division of Pulmonology, 1st Department of Medicine, University of Pecs, School of Medicine, 13 Ifjusag, Pecs H-7624, Hungary

[2] Department of Pathology, University of Pecs, School of Medicine, Pecs, Hungary

Introduction

Lung cancer is a leading cause of cancer-related mortality worldwide [1, 2] and smoking plays an important role in the development of the disease [3]. In Hungary, lung cancer stands at the 1st place of cancer-related mortality in men and at the 2nd in women. According to the histology, we can distinguish two main groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC is more chemo- and radiosensitive than NSCLC [4].

In the last decades many efforts have been made to find new therapeutic targets for the treatment of NSCLC. Tyrosine kinase inhibitors inactivate receptor (EGFR, HER2 and ALK) or non-receptor tyrosine kinases (Src, Abl, Syk family) [5]. Epidermal growth factor receptors (EGFRs) are the members of the ErbB receptor tyrosine kinase family. They consist of an extracellular ligand binding domain, a transmembrane, a juxtamembrane, a tyrosine kinase and a cytoplasmic domain. Upon ligand binding, dimerization of the receptors occurs that leads to autophosphorylation of tyrosine residues. These phosphorylation events trigger further downstream signaling molecules of the EGFR pathways [6, 7]. EGFRs and their mutants play an important role in various cancers including lung cancer. EGFRs act as new therapeutic targets inhibited by antibodies binding to the extracellular domain of the receptor. Moreover, small molecule inhibitors called tyrosine kinase inhibitors can directly block kinase activation [8, 9]. Erlotinib, gefitinib and afatinib are TKIs used in the treatment

of NSCLC. Erlotinib and gefitinib reversibly bind to the ATP binding site and inhibit the autophosphorylation of the EGFR kinase [10, 11]. Afatinib irreversibly inhibits the EGFR; it covalently binds to the 797 cysteine residue of the receptor [12].

First erlotinib clinical trial (BR21) related to lung cancer was published in 2005 [13]. According to this study, erlotinib can prolong the progression free survival (PFS) in patients with NSCLC following first-line or second-line chemotherapy. The response rate was especially high among non-smoking Asian women with adenocarcinoma [13]. Simultaneously, others reported that certain EGFR mutations are responsible for the TKI efficiency [11, 14]. EGFR mutations are present in about 15 % of NSCLC in Caucasian and 50 % in the Asian population [15, 16]. Exon 19 deletion (50 %) and exon 21 L858R point mutation (40 %) of EGFR are the two most common mutations. However, rare mutations such as exon 20 insertions, G719X, L861Q mutations also occur [11, 14, 15].

TKIs as first-line treatment were also initially proved in Asian population. Non- or former light smokers were involved in these studies. EGFR mutation status was an important aspect of the trials. In the IPASS study, the 12 month-long PFS was significantly higher in gefitinib-treated EGFR mutants compared to those receiving carboplatin and paclitaxel [17, 18]. In the First-SIGNAL study, non-smoker Korean patients were involved. PFS was also higher in the gefitinib-treated group than in those receiving cisplatin and gemcitabine [19]. In the US, the CALGB 30406 study was performed on non-smoking Caucasians with EGFR mutations. Erlotinib treatment was compared to erlotinib plus carboplatin and paclitaxel. No significant difference was found in the PFS or overall survival (OS) of these 2 groups [20]. In the OPTIMAL study, erlotinib versus carboplatin and gemcitabine were used in Chinese EGFR mutated NSCLC population. The PFS was significantly higher in the erlotinib group [21]. The EURTAC study was performed in European NSCLC patients bearing EGFR mutation. Erlotinib was compared to platinum-based doublet (cis-or carboplatin) plus gemcitabine or docetaxel. PFS was also significantly higher in the erlotinib group [22]. The LUX-Lung3 study compared afatinib treatment to cisplatin and pemetrexed in Asian and European population with EGFR mutation, with significantly positive outcome on PFS in the afatinib group [23]. Similar results were found in the LUX-Lung 6 study on EGFR-mutant Asian NSCLC patients treated with afatinib versus cisplatin and gemcitabine [24]. OS did not increase significantly upon EGFR-TKI treatment in any of the aforementioned observations except in cases with EGFR mutation 19 deletion treated by afatinib [25]. According to the results of above-mentioned studies, the ESMO guideline recommends EGFR mutation analyses in all patients with NSCLC, except in those with squamous subtype. In the case of squamous subtype, EGFR mutation analysis is only recommended if the patient is a non-smoker or former light smoker (<15 packs/year). In case of patients with activating EGFR mutation, TKIs should be considered as first-line therapy [26].

In our recent work, we analyzed our patient dataset with EGFR mutated lung adenocarcinoma.

Patients and Methods

In the last 6 years (Jan. 2009-Aug. 2015), 446 patients with lung adenocarcinomas were screened for EGFR mutations at the Division of Pulmonology, University of Pecs and 44 subjects were found positive (9.86 %).

The EGFR mutational analysis was performed on both cytology samples (pleural fluid, bronchial brush biopsy, transbronchial needle aspiration and transthoracic aspiration specimens) and formalin fixed paraffin-embedded tissue blocks. Following DNA extraction, exon 19 and exon 21 of EGFR were amplified by polymerase chain reaction (PCR) using the following primers: exon 19 forward primer (ex19-S1): 5′-ATCCCAGAAGGTGAGAAAGATAAAATTC-3′, reverse primer (ex19-AS1): 5′-CCTGAGGTTCAGAGCCATGGA-3′, exon 21 forward primer (ex21-S1): 5′-CAGCCAGGAACGTACTGGTGA-3′, reverse primer (ex21-AS1): 5′-TCCCTGGTGTCAGGAAAATGCT-3′. The PCR conditions were set as described by Asano et al. [27]. The mutated (deleted) and wild type exon 19 products were separated by fragment length analysis. The sensitivity of this method was estimated to be 3–5 %.The PCR product using the exon 21 primers were Sanger sequenced following purification to detect point mutations. The sensitivity of the method is 15 % mutated alleles. Expert pathologist evaluated the tumor cell ratio, the sample was rejected for EGFR testing below 30 %. Due to the Hungarian guideline, tumor cell-normal cell ratio in the obtained tissue, as well as the absolute tumor cell number have great significance, which information must be provided in the primary lung cancer diagnosis [28].

Data of these patients were analyzed retrospectively. Gender, smoking behavior, type of EGFR mutation and EGFR TKI treatment were evaluated. In patients receiving EGFR TKI treatment, disease control rate (DCR), PFS, primary resistance and radiological progression were also determined.

Statistical analysis was performed using the IBM SPSS Statistics Version 22.0 (SPSS, Inc., Chicago, IL, USA) software. Kolmogorov-Smirnov test was used to determine the distribution of the data. Normally distributed data are presented as mean ± SE. Relationships between binomial variables were tested using Chi-square and Fischer's exact tests as appropriate. Ratio scaled variables of subgroups were compared using Student's t-test. The determinants of DCR, 6-month and 12-month PFS were investigated by binary logistic regression

analysis using backward method. Significant results were those with P values <0.05.

Results

The age of the 44 patients with EGFR-mutated lung adenocarcinoma was 69.6 ± 1.6 (40–89) years. The female/male ratio was 31/13 (Fig.1a). Men were significantly younger than women: 62.9 ± 2.4 versus 72.1 ± 2.0 years ($p = 0.008$). Non-smokers represented the 77.2 % of the patient population, 4 patients were former smokers and 6 cases were smokers at the time of the diagnosis (Fig. 1b).

Focusing on the EGFR mutations, the exon 19 deletion was detected in 61.4 % of the patients, while L858R point mutation in exon 21 was observed in 34.1 % of them. In one subject, both exon 19 and 21 mutations were detected simultaneously. A rare mutation located in exon 21 was found in another patient (Fig.1c).

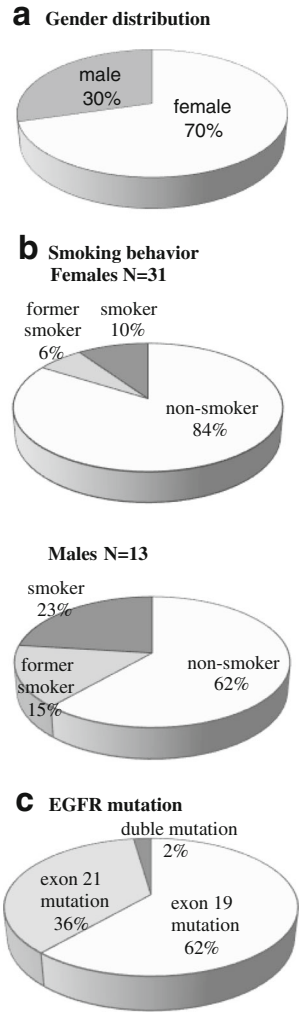

Fig. 1 Data of 44 adenocarcinoma patients with EGFR mutation were analyzed according to a: gender, b: smoking behavior, c: type of the EGFR mutation

Until the end of the observation period, 38 patients received EGFR-TKI treatment; twenty-nine patients were treated with erlotinib, nine received gefitinib (Fig. 2a). One patient progressing after 20 months of erlotinib treatment got afatinib in a clinical trial. TKI treatment was started as 1st, 2nd and 3rd line treatment in 22, 11 and 5 cases, respectively (Fig. 2b.). The efficacy of TKI treatment was first evaluated after 2 months. Data were available in 35 cases, because three patients died within 2 months. Primary resistance to the TKI treatment was diagnosed if disease progression was detected at the 2-months visit; it was found in five cases (14.3 %). The beneficial effect of TKI treatment was established in 30 patients, the disease control rate was 85.7 % (Fig.2c.). One patient responded by complete remission; partial response and stable disease were detected in 16 and 13 cases, respectively. No difference was found between groups of patients responding with complete or partial remission and stable disease in age, gender, mutation type, smoking behavior, type and sequence of TKI therapy. The progression free survival of the 35 patients was 12.4 + 2.1 months, the longest PFS was 54 months and this patient still receives TKI treatment. Six patients received TKIs for less than 6 months at the time of

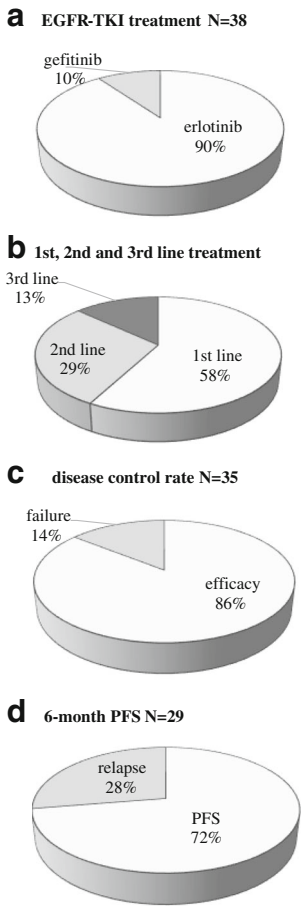

Fig. 2 Out of 44 patients 38 received EGFR-TKI treatment. a: the type of EGFR-TKI therapy, b: the sequence of treatment, c: disease control rate, d: the PFS over 6 months

closing database, 21 patients had PFS beyond six months (72.4 %) (Fig.2d.) and 11 from 22 patients survived without progression over 12 months (50 %). PFS beyond six months were significantly more common in non-smokers compared to smokers and former smokers ($p = 0.005$); age, gender and the type of mutation did not influence the efficacy of TKI treatment and the duration of PFS. Gefitinib treatment seemed to be more efficient based on the 12-month PFS ($p = 0.045$). The small number of cases may limit the validity of interpretation. The independent determinants of therapeutic response were further assessed in regression models. Age, gender, type of mutation, smoking behavior, the sequence of TKI therapy and the type of TKI were included into these analyses. The sequence of TKI therapy was an independent determinant of the therapeutic response ($p = 0.046$); DCR was higher in patients treated in first line (94.7 %) than in second and third line (81.8 and 60.0 %). The detrimental effect of smoking and former smoking was confirmed on the 12-months PFS. Gefitinib treatment was found to be an independent factor of 12-month PFS. The models are summarized in Table 1.

Discussion

In the last 6 years, the EGFR mutation analysis became available at our department. Among the 446 screened patients with lung adenocarcinoma, 44 were positive for EGFR mutations. This 9.86 % ratio of EGFR positivity is lower than it was previously reported in the Caucasian population [15, 16]. Participating in the CEETAC study, 92 further patients were screened and positivity was found in 7 cases (7.6 %), confirming the lower incidence of EGFR mutations in our patient population [unpublished data]. In the largest Hungarian cohort published until now, the classic EGFR mutant cases represented only 5 % [29].

Only data of patients with adenocarcinoma were analyzed, since unfortunately, the National Health Insurance Fund of Hungary gives support for TKI treatment only for these patients and not for all NSCLC [26]. Despite of the small number of the patients in our center, these findings are in accordance with the international studies [17–24]. Non-smokers with EGFR mutated adenocarcinoma had the highest benefit from TKI treatment. Furthermore, none of the current smokers at the time of diagnosis had PFS over 6 months. PFS over 6 and 12 months was achieved in 72.4 % and 50 % of patients, independently from age, gender and type of mutation. It is important to note that male gender was not disadvantageous in our patient population.

Beside secondary resistance that develops during the treatment, primary/intrinsic resistance against TKIs may occur with a frequency of 4–10 % in NSCLC patients having EGFR mutations [30]. The definition of primary resistance varies among authors which is either progressive disease or stable disease without remission as the best response given to the TKI therapy [31]. The first definition was used in our study and 14.3 % of patients were found not responding to the TKI therapy. The exact mechanisms of primary resistance are still unclear. It might be caused by insertions in exon 20 of EGFR, mutations of KRAS, loss of PTEN, BRAF mutations, and increased protein levels of MAPK, ABCG2, IFGR1, BCL-2 and mediators of angiogenesis [32–34]. Host (cigarette smoking, BIM expression) and tumor-dependent factors (EGFR mutation, mutation in the EGFR signaling pathway) might also be responsible for the development of resistance [31]. Others distinguish various primary resistance mechanisms in EGFR wild type (other pathways are responsible for the tumor development and growth: (ROS1, RET, BAF) and EGFR mutated (compensatory pathways) adenocarcinomas [35]. According to Carretera et al., three primary EGFR TKI resistance mechanisms exist: KRAS mutation, loss

Table 1 Independent determinants of therapeutic response to EGFR TKI therapy in lung adenocarcinoma patients with mutated EGFR – binary linear logistic regression models

	Variables	Independent determinants	*p* value	Cox & Snell R^2	Nagelkerke R^2
Disease control rate	Age	Sequence of TKI therapy	0.045	0.100	0.179
	Gender				
	Non-smoking				
	Type of EGFR mutation				
	Sequence of TKI therapy				
	Type of TKI				
12-month PFS	Age	Non-smoking	0.052	0.359	0.478
	Gender				
	Non-smoking	Type of TKI	0.014		
	Type of EGFR mutation				
	Sequence of TKI therapy				
	Type of TKI				

of PTEN and concurrent T790 M mutation in EGFR [36]. Additionally, others have shown the amplification of ErbB family members besides the biased KRAS and PTEN as another contributor in the primary resistance [37]. None of these factors were investigated in our patients. However, it is worth to mention that the therapeutic response was better if the TKI therapy was used in first line; primary resistance was more common after previous chemotherapy.

The definition of secondary or acquired resistance was determined by Jackman and colleagues [38]. According to this, patients need to meet the following criteria: they should have been treated with EGFR TKI, the tumor should bear activating EGFR mutations (G719X,exon 19 deletion, L858R,L861Q) and/or clinical benefit from previous EGFR TKI treatment (partial or complete remission or >6 months PFS) and systemic progression of the disease while being on EGFR TKI treatment within at least 30 days [38]. Two frequent mutations have been proved to be responsible for secondary resistance: T790 M mutation and MET amplification [39–41]. Other rare mutations have also been determined to be responsible for TKI resistance: D761Y, T854 A, L747S, EGFR amplification and mutations in the PIK3CA [42–45].

In summary, the frequency of EGFR mutation in a Hungarian group of patients with lung adenocarcinoma is 9.86 %, lower than it was previously published in Caucasian populations. The distribution of mutations was similar to the literature data. TKI treatment of these patients is reassuring since 85.7 % of cases had benefit from TKI therapy. First line treatment seems to be the best option. Smoking habit decreases the probability of the EGFR mutant status but does not exclude it. Male patients responded similarly to women in our study. The potential advantage of gefitinib versus erlotinib might be confirmed in further investigation.

Before starting first line treatment, the EGFR mutation status should be examined in all IIIB and IV clinical stage lung adenocarcinomas, independent from the smoking habit and gender.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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