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EGFR MUTATIONS STATUS FOR NON-SMALL-CELL LUNG CANCER PATIENTS

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Key words: non-small-cell lung cancer (NSCLC), targeted therapies, tyrosine kinase inhibitors, EGFR mutation, cfDNA.

Abbreviations: NSCLC - Non-small cell lung cancer; EGFR - Epidermal growth factor receptor; TKI - Tyrosine kinase inhibitor; FFPE - Formalin-fixed, paraffin-embedded; PFS - Progression-free survival; cfDNA - Circulating cell-free nucleic acids; ctDNA - Circulating tumor nucleic acids

Summary

With the advent of the molecular-targeted therapy, rapid progress has been made in the treatment of advanced or recurrent non-small-cell lung cancer (NSCLC). EGFR mutations detection in tumor is important to determine an appropriate treatment of EGFR TK inhibitors.

We investigated *EGFR* mutation status for patients with non-small cells lung carcinoma (NSCLC) patients' tumorous cells from FFPE material in Klaipeda University Hospital, Lithuania (mutation test for the qualitative detection and identification of mutations in exons 18, 19, 20 and 21 of the EGFR gene) and T790M mutation in cfDNA (Liquid biopsy).

Mutations of EGFR from FFPE were detected in 24 of the 119 patients (20%). *EGFR* mutations were more frequently found in women (13 of 36, 36%). cfDNA (Liquid biopsy) testing results show one of the patients had *EGFR* 20 exon T790M mutation detected which is resistance determining factor for the first and second generation EGFR TK Inhibitors treatment and a predicting marker for the third generation EGFR TK Inhibitors treatment.

Introduction

Lung cancer is the most commonly diagnosed cancer as well as the leading cause of cancer-related deaths in the world [1]. Non-small cell lung cancer (NSCLC) accounts for approximately 80% cases of lung cancer [2]. Most NSCLC patients are diagnosed with advanced or distant stages and they are ineligible for curative surgery and often suffer a poor survival. Identifying biomarkers related to treatment response and prognosis may be helpful to improve the clinical outcome of patients with NSCLC. The developments of molecular pathology methods have become increasingly important in the prediction of chemotherapy sensitivity and mutation analysis to identify driver mutations as important targets of new therapeutic agents. The most significant changes in the treatment of NSCLC revealed in new pathologic classification and in the introduction of molecularly targeted therapies, which include monoclonal antibodies and small molecule tyrosine kinase inhibitors [3]. In the past decade, significant improvements have been made due to the development of targeted therapies, such as EGFR tyrosine kinase inhibitors (TKIs), for advanced NSCLC. Patients with a sensitizing exon 19 deletion or an exon 21 substitution mutation are highly responsive to first-generation EGFR TKIs, such as gefitinib and erlotinib, compared to traditional platinum-based doublet chemotherapy, with a prolonged time to progression or improved progression-free survival (PFS) without serious drug-specific side effects.

The side effects of these agents are generally better tolerated than those of conventional chemotherapy and show higher efficacy. The most important factor follows: histology subtypes, gene mutation status, patients' selection, drug toxicities and occurrence of drug resistance. A couple years ago a median lung cancer survival rate was 10–12 months. Since specific molecular targets are available, there is a significant increase in median survival rates up to 24–36 months. This gives an opportunity to provide a new standard of care [3]. Detection of *EGFR* mutations in tumor is very important in determining an appropriate treatment of EGFR TKI. *EGFR* wild-type tumors have a shorter progression survival rate than those with an *EGFR* mutation [4].

The use of circulating cell-free nucleic acids (cfDNA), consisting circulating cell-free (tumoral) DNA (cfDNA-ctDNA)), as a liquid biopsy in lung cancer offers opportu-

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nities to detect resistance mechanisms, such as the *EGFR* T790M mutation in the case of EGFR TKI use, at an early stage and to detect resistant mutation for *EGFR* mutated patients acquired resistance to the first- and second-generation EGFR TKI.

Objective: to evaluate *EGFR* gene mutations frequency for Non-Small-Cell Lung Cancer patients in Klaipeda University Hospital.

Materials and methods

Samples: formalin-fixed, paraffin-embedded (FFPE) tumor samples were obtained from and diagnosed NSCLC for 119 patients with NSCLC diagnosis from Klaipeda University Hospital.

DNA was extracted from sections of tissues FFPI tumour cells. DNA was extracted using Qiagen DNA FFPE tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

The *EGFR* mutation qualitative detection and identification of mutations were accomplished using therascreen *EGFR* Pyro Kit in exons 18, 19, 20 and 21 of the *EGFR* gene Kit, (Qiagen, Hilden, Germany).

Circulation tumor cells from plasma were extracted with QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) and these cells *EGFR* mutation (19 exon's deletions, 20 exon's T790M and 21 exon's L858R) were detected using *therascreen EGFR* Plasma RGQ PCR Kit (Qiagen, Manchester, UK).

Results

EGFR mutations were detected in 24 (20%) out of 119 samples, while the rest of the samples did not have *EGFR* mutations.

Table 1. DNA samples from NSCLC patients were tested for *EGFR* mutations

	Mutation +	**Mutation -**	**Total**
Female	13	23	36 (30%)
Male	11	72	83 (70%)
Total	24 (20%)	95 (80%)	119 (100%)

30% female and 70% male patients' DNA were tested. *EGFR* mutations detected more frequently in females compared to males (36% female and 13% male were *EGFR* positive) (Table 1.)

The *EGFR* mutation frequency in Caucasian NSCLC patients was reported to be 10-15% [5]. A recent study reviewed *EGFR* mutation incidence in European countries [6,7]. *EGFR* mutation frequency in Europe ranged from 6% (Switzerland) to a maximum of 37.5% (Germany), depending on ethnicity and patient's characteristics.

Hatson I et al. accomplished research results were different compared to our results – we have detected more mutations in female and male population (approximately 3 times more female and 4 times male population), however, identified mutations frequency was similar in both researches (the most common mutations were 19 exon deletion and 21 exon L861Q mutation) [8].

De Grève J et al. accomplished research was similar compared to our results – *EGFR* mutations detected in approx. of 20% in both investigate populations [9].

The most frequent mutations were 19 exon deletion and 21 exon L681Q mutations. These two mutations constitute 62,5% out of all detected mutations (Table 2.). These patients had Second Generation EGFR TKI treatment.

Two patients were testing for T790M mutation status (NSCLC heterogeneous tumor had been suspected), because progression of the disease during treatment with the EGFR TK Inhibitors. Circulating tumor DNA (ctDNA) is the cell-free DNA released from dying cancer cells [10], and represents an emerging field of cancer research [11].

One of the patients had *EGFR* 20 exon T790M mutation detected which is resistance determining factor for the EGFR TKI treatment and a predicting marker for the Third Generation EGFR TKI treatment. T790M is a frequently reported secondary mutation of the *EGFR* gene and is detected in 60% of patients with acquired resistance to EGFR TKI therapy [12].

Discussions

Nowadays, EGFR-TKIs are the most successful example of targeted therapy in NSCLC. *EGFR* gene mutations are the standard biomarkers for selecting NSCLC patients to receive EGFR-TKIs treatment. *EGFR* mutations can be

Table 2. *EGFR* mutations distribution

	G719X (Exon 18)	**G719A (Exon 18)**	**Exon 19 deletion**	**S768I (Exon 20)**	**L858R (Exon 21)**	**L861Q (Exon 21)**	**Total**
Female	0	1	5	1	5	1	13
Male	1	2	2	1	3	2	11
Total	1 (4,2%)	3 (12,5%)	7 (29,2%)	2 (8,3%)	8 (33,3%)	3 (12,5%)	24 (100%)

classified into three major categories: in-frame deletion in exon 19, insertion mutation in exon 20, and missense mutations in exon 18–21 and only these mutations are association with lung cancer treatment. The most frequent mutations were located in exon 19 and exon 21 [3]. *EGFR* mutations are generally associated with sensitivity to TKI therapy (Second Generation EGFR TKI), while *EGFR* mutation G719M is resistance mutation to Second Generation EGFR TKI. Patients whose biopsies tested negative for T790M and had no response to treatment, may have been due to tumor heterogeneity [13]. T790X mutation is sensitive to Third Generation EGFR TKIs, but this has to be proved by a circulation tumor cells test.

In Lithuania all patients who are diagnosed with NSCLC are tested for *EGFR* gene mutations, which are linked to the EGFR TKIs treatment. College of American Pathologists (CAP), International Association for Lung Cancer (IASLC) and the Association for Molecular Pathology (AMP) guide-lines are similar to Lithuanian, but they recommend additionally doing molecular marker for NSCLC treatment - ALK rearrangement.

EGFR mutation testing and ALK rearrangement status are the only two molecular markers considered standard of care for NSCLC treatment and are the subject of CAP, International IASLC and AMP Guide-lines published in 2013 [14]. *EGFR* mutation and ALK rearrangement testing are also a feature of the Version 1.2014 National Comprehensive Cancer Network (NCCN) clinical practice guidelines for Non-Small Cell Lung Carcinoma [15] and KRAS mutation testing asa potential prognostic marker for NSCLC [16,17].

It makes assumptions for better patients' treatment and optimizes treatment options – personalized medicine. Molecular lung cancer profile knowledge allows developing new diagnostic methods and targeting therapy instruments. One of the newest target therapy instruments is the third generation EGFR TKI treatment form assigned to occurring *EGFR* gene 20 exon T790M resistance mutations to second generation EGFR TKI treatment. Therefore, if patients presently treated with the second generation EGFR TKI treatment and it treatment was unsuccessful are tested for resistance mutations, because tumor can be heterogeneous.

Various driver mutations blocking drugs for lung cancer treatment are being developed - research is in different clinical testing stages [9,13], some of the drugs are already adopted in treatment (*EGFR*, *ALH, KRAS, BRAF*), therefore, based on molecular profile knowledge, we are planning to test 20-56 oncogenes for driver mutations. In the near future lung cancer treatment sequence will be as follows: pathological diagnosis, driver mutations detection from FFPE, new generation sequencing (20-56 oncogenes panel), molecular profile analysis and then results will determine the best method of lung cancer treatment.

Targeted therapy based on molecular characterizations has greatly influenced the treatment strategies in NSCLC. Gene mutation analyses are the commonly used predictive biomarkers for selecting NSCLC patients to receive targeted agents. However, the current mutation analyses are often based on tumor tissues and have many limitations [18]. First, the accessibility of tumor tissues is not always satisfactory as most NSCLC patients are diagnosed with advanced stages and unsuitable to provide tissues through invasive surgery or biopsy. Second, surgery and biopsy are not without clinical complications. The adverse events rate for thoracic biopsy was reported to be approximately 20% [18]. Furthermore, some percentages of NSCLC patients will develop resistance to molecular-targeted agents [20].

Reference

1. Travis WD, Brambilla E. et al. International association for the study of lung cancer/American thoracic society/European respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thoracic Oncol 2011; 6:2244–2285. http://dx.doi.org/10.1097/JTO.0b013e318206a221
2. Mino-Kenudson M. et al. A novel, high sensitive antibody allows for the routine detection of ALK – rearranged lung adenocarcinomas by standard immunohistochemistry. Clin Cancer Res 2010; 16:1561–1571. http://dx.doi.org/10.1158/1078-0432.CCR-09-2845
3. Bittner N, Ostoros G, Géczi L. New treatment options for lung adenocarcinoma – in view of molecular background. Pathol. Oncol. Res, 2012.
4. Mok TS, Wu Y, Thongprasert S, Yang C-H, Chu D-T, Saijo N. et al. Gefiti-nib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57. http://dx.doi.org/10.1056/NEJMoa0810699
5. Chougule A, Prabhash K, Noronha V, Joshi A, Thavamani A, Chandrani P, Upadhyay P, Utture S, Desai S, Jambhekar N. et al: Frequency of EGFR mutations in 907 lung adenocarcinoma patients of Indian ethnicity PLoS One 8, 2013. http://dx.doi.org/10.1371/journal.pone.0076164
6. Szumera-Ciećkiewicz A, Olszewski WT, Tysarowski A, Kowalski DM, Głogowski M, Krzakowski M, Siedlecki JA, Wągrodzki M and Prochorec-Sobieszek M: EGFR mutation testing on cytological and histological samples in non-small cell lung cancer: A Polish, single institution study and systematic review of European incidence. Int J Clin Exp Pathol 2013; 6:2800-2812.
7. Papadopoulou E. et al. Determination of EGFR and KRAS mutational status in Greek non-small-cell lung cancer patients. Oncology letters 2015;10: 2176-2184.

http://dx.doi.org/10.3892/ol.2015.3600

8. Hantson I. et al. Performance of standard procedures in detection of EGFR mutations in daily practice in advanced NSCLC patients selected according to the ESMO guideline: a large Caucasian cohort study. Translational Respiratory Medicine 2014; 2:9.
   http://dx.doi.org/10.1186/s40247-014-0009-0
9. De Grève J. Prospective evaluation of first-line erlotinib in advanced non-small cell lung cancer (NSCLC) carrying an activating EGFR mutation: a multicenter academic phase ii study in caucasian patients (FIELT). PLOS ONE 2016.
   http://dx.doi.org/10.1371/journal.pone.0147599
10. Schwarzenbach, H., Hoon, D. S. & Pantel, K. Cell-free nucleic acids as biomarkers in cancer patients. Nature Review Cancer 2011; 11: 426–437.
    http://dx.doi.org/10.1038/nrc3066
11. Kato K. at al. Numerical indices based on circulating tumor DNA for the evaluation of therapeutic response and disease progression in lung cancer patients. Scientific Reports 2016; 6:29093.
    http://dx.doi.org/10.1038/srep29093
12. Wang J. et al. Intrinsic resistance to EGFR tyrosine kinase inhibitors in advanced non small-cell lung cancer with activating EGFR mutations. OncoTargets and Therapy 2016; 9: 3711–3726.
    http://dx.doi.org/10.2147/OTT.S106399
13. Bittner N, Ostoros G, Géczi L. New treatment options for lung adenocarcinoma – in view of molecular background. Pathol. Oncol. Res, 2013.
14. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G. et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Patho. Arch Pathol Lab Med 2013;137:828–60.
    http://dx.doi.org/10.5858/arpa.2012-0720-OA
15. National Comprehensive Cancer Network. NCCN Clinical practice guidelines in oncology, non-small-cell lung cancer version 1.2014; 2013.
16. Mascaux C, Iannino N, Martin B, Paesmans M, Berghmans T, Dusart M. et al.The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer 2005;92:131–9.
    http://dx.doi.org/10.1038/sj.bjc.6602258
17. Slebos RJ, Kibbelaar RE, Dalesio O, Kooistra A, Stam J, Meijer CJ. et al. K-rasoncogene activation as a prognostic marker in adenocarcinoma of the lung. NEngl J Med 1990; 323:561–5.
    http://dx.doi.org/10.1056/NEJM199008303230902
18. Overman MJ, Modak J, Kopetz S. et al. Use of research biopsies in clinical trials: are risks and benefits adequately discussed? J Clin Oncol 2013; 31:17-22.
    http://dx.doi.org/10.1200/JCO.2012.43.1718
19. Janne PA. Challenges of detecting EGFR T790M in gefitinib/erlotinib-resistant tumours. Lung Cancer 2008; 60:S3-9.
    http://dx.doi.org/10.1016/S0169-5002(08)70099-0
20. Russo A, Franchina T, Ricciardi GR. et al. A decade of EGFR inhibition in EGFR-mutated non small cell lung cancer (NSCLC): Old successes and future perspectives. Oncotarget 2015; 6:26814-26825.
    http://dx.doi.org/10.18632/oncotarget.4254

EGFR MUTACIJŲ STATUSAS TARP PACIENTŲ, SERGANČIŲ NESMULKIALĄSTELINE PLAUČIŲ KARCINOMA

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Raktažodžiai: nesmulkialąstelinė plaučių karcinoma, taikininė terapija, tirozinkinazės inhibitoriai, EGFR geno mutacijos.

Santrauka

Tikslas: Įvertinti EGFR geno mutacijų dažnius pacientams, sergantiems nesmulkialąsteline plaučių karcinoma Klaipėdos universitetinėje ligoninėje.

Molekulinės-taikininės terapijos atsiradimas padarė proveržį gydant nesmulkialąstelinį plaučių vėžį. Naviko EGFR mutacijų nustatymas yra svarbus parenkant taikininę EGFR terapiją.

Klaipėdos universitetinėje ligoninėje iš FFPI ėminių mes nustatome pacientų, sergančių nesmulkialąsteliniu plaučių vėžiu, navikų EGFR mutacijų statusą (tiriame 18, 19, 20 ir 21 egzonų mutacijas), bei 20 egzono T790M mutaciją cirkuliujančioje DNR (skysta biopsija).

FFPI ėminiuose EGFR mutacijos buvo nustatytos 24 pacientams iš 119 (20%). EGFR mutacijos dažniau nustatytos moterims (13 moterų iš 36, tai sudaro 36%). Taip pat nustatyta vienam iš dviejų pacientų EGFR 20 egzono T790M mutacija cirkuliuojančiose DNR (skystoje biopsijoje). Ši mutacija yra resistentiškumo faktorius pirmos ir antros kartos EGFR taikininei terapijai, taip pat predikcinis markeris trečios kartos EGFR taikininei terapijai.

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