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Detection of somatic EGFR mutations in patients with non-small cell lung cancer

Relevance: *EGFR mutations can serve as a clinical marker that allows predicting and evaluating the efficiency of non-small cell lung cancer (NSCLC) therapy with tyrosine kinase inhibitors. The increased enzyme activity of EGFR due to mutations is a determining factor in cancer development, including NSCLC. Study of the mutations allows establishing a link between genome alterations and NSCLC development.*

Purpose of the study: *Determination of frequency and spectrum of EGFR mutations for justification of choice of target therapy with tyrosine kinase inhibitors. Study of mutation frequency related to gender and other demographical parameters of the target population.*

Results: *138 NSCLC patients were included in the study of frequency and spectrum of gene mutations. Their post-operational or biopsy material was delivered from Almaty or regional oncological dispensaries to the Center for Morphological Research of Kazakh Institute of Oncology and Radiology (KazIOR) or they were treated in KazIOR during 2017. About 78% of the studied patients had no common EGFR exon 21 or exon 19 mutations. The most common was the EGFR exon 21 mutation – 10.14%, exon 19 deletion was detected in 7.25%, with 1 (0.72%) case of exon 20 insertion.*

Conclusion: *The lack of the mentioned mutations indicates the presence of possible unstudied mutations. The introduction of new target medications into clinical practice necessitates the study of molecular-genetic profile of the tumor to implement personalized approach to treatment.*

Keywords: *lung cancer, EGFR, mutation frequency, non-small cell lung cancer (NSCLC), tyrosine kinase inhibitors (TKI).*

Introduction. EGFR is the gene that encodes the epidermal growth factor receptor. It is responsible for cells signals and the attachment of specific ligands [1]. EGFR is expressed in many tissues, including the skin, placenta, and thyroid. It is placed on the short arm of chromosome 7 (Figure 1). This gene is responsible for transmembrane glycoprotein from the family of protein kinases, that is, it is located on both external and internal sides of the plasma membrane [2]. Such location allows the EGFR to form dimers from the outside of the membrane and to transmit signal in the cell cascade by tyrosine kinase, located on the cytosolic side of the plasma membrane [3].

This gene triggers a series of cascades responsible for changing the expression of other key genes in the cell. Unlike other genes, this gene has only 7 specific ligands, and it determines its targeted action in the cell.

Genetic mutations in EGFR were found mainly in the domain responsible for enzymatic activity of tyrosine kinase [4]. The most common mutations are small deletions in the reading frame, deletion of exon 19, and deletion of exon 21 (L845R) (Figure 2).

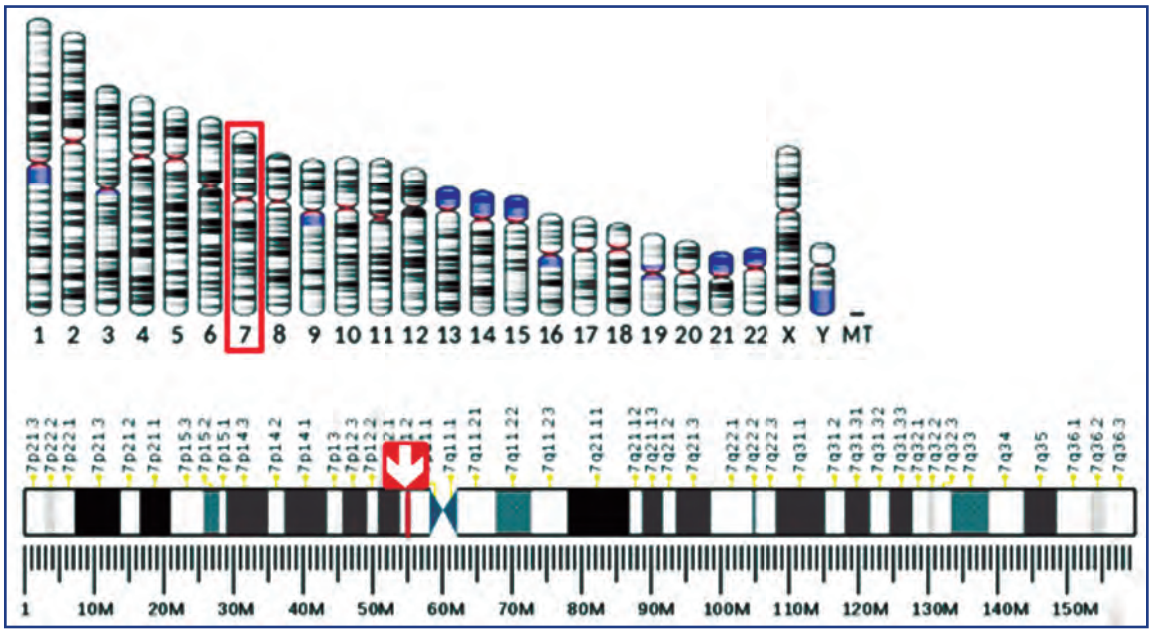

Figure 1 - Location of EGFR gene on chromosome 7.

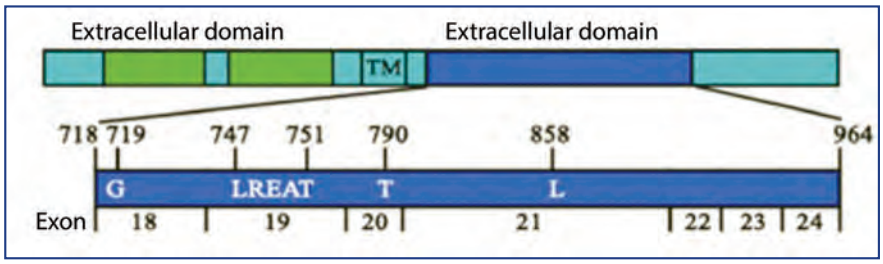

Figure 2 – Scheme of the EGFR receptor and the position of the major mutations [5].

These mutations explain the relevance of studying EGFR in cancer development as they affect the ATP adherence site leading to a 50-fold increase in the enzyme activity. This increased activity inhibits the signals for apoptosis and increases the signals for survival. At that, the cells subject to safe removal, apoptosis, survive and proliferate. With the accumulation of mutations, the cells gradually acquire the properties of cancer cells, as well as the ability to migrate and cause the proliferation of neighboring, healthy cells. These genetic mutations are irreversible and persist throughout the life of the individual.

The most common drugs for the treatment of cancer caused by EGFR gene mutations are tyrosine kinase inhibitors (TKI). Gefitinib was the first drug synthesized and approved in that line, followed by Erlotinib and Afatinib [6]. Those drugs selectively displaced ATP, the tyrosine kinase substrate occupying the active site of the enzyme with its struc-ture. It prevented phosphorylation of the cytosolic part of the protein and blocked the transmission of signal through the cascade. Those drugs reduced the proliferation and survival of cancer cells, increased apoptosis and improved ef-ficiency of chemotherapy and radiotherapy. In other words, the efficacy of therapy is improved only by combination of drug therapy and other therapies. Drugs of the second or third generation are used In case of resistance due to secondary mutations. The comparative efficacy analysis of different TKIs substantiates the need for an individual ap-proach in the choice of treatment due to differences in the course of the disease in different patients.

Today, the EGFR mutations are an essential clinical marker which allows predicting and assessing the efficacy of NSCLC therapy with TKI [7]. Purpose of the study was to analyze and characterize the EGFR mutations in Kazakhstani population. This study allows determining the role of mutations in NSCLC as they serve as a justification for the choice of targeted therapy. For this reason, TKIs in the treatment of NSCLC are prescribed only after the EGFR mutation is de-tected. Since TKI has become a part of the protocol of treating NSCLC in Kazakhstan, it is important to assess the fre-quency and range of EGFR genes mutations in Kazakhstani patients.

Materials and methods. The study included 138 patients with NSCLC whose postoperative or biopsy material was deliv-ered from Almaty Regional Cancer Center to the Center for Morphological Studies of Kazakh institute of Oncology and Radiology (KazIOR) or they were treated at KazIOR during 2017. Most of them (60.87%) were men. The average age of men and women was almost the same – 60.5 and 59.9 years, respectively. 68.84% of the included patients were from Almaty, 13.04% - from Karaganda, the rest – from other 10 regional centers of Kazakhstan (Table 1).

Table 1 – Characteristics of the group of patients with NSCLC who underwent genetic testing

Parameter	Number of patients	
	abs	%
Sex		
Men	84	60,87%
Women	54	39,13%
Average age, years		
Men	60.5	
Women	59.9	
Admitted from		
Aktau	3	2.17%
Aktobe	1	0.72%
Almaty	95	68.84%
Astana	6	4.35%
Karaganda	18	13.04%
Kostanay	3	2.17%
Semey	1	0.72%
Taldykorgan	2	1.45%
Taraz	1	0.72%
Uralsk	1	0.72%
Ust-Kamenogorsk	4	2.90%
Shymkent	3	2.17%

The material for the analysis included tumor cells from paraffin blocks containing postoperative and biopsy mate-rial. DNA from tumor cells fixed in formalin and enclosed in paraffin blocks (FFPE) was extracted by liquid-phase method using BioLink set for DNA extraction in accordance with the manufacturer's instructions. To detect muta-tions, DNA was amplified by real-time PCR using the sets of reagents for determining the EGFR mutations from Bi-oLink (exon 21: L858r, exon 19: deletions) and Roche (exon 18: G719X (G719A, G719C, and G719S), exon 19: dele-tions and complex mutations, exon 20: S768I, T790M, and insertions, exon 21: L858R and L861Q).

Results and discussion. In the examined cohort of patients, most of the samples, 108 (78.26%), had no EGFR mutation. The L858R mutation of exon 21 was the most frequent – 14 (10.14%) patients, with less frequent dele-

tions of exon 19 – 10 (7.25%) patients, and 1 (0.72%) case of insertion of exon 20. 3.62% samples were not suitable for analysis, mainly due to DNA degradation or insufficient number of tumor cells in the sample (table 2).

Table 2 - Spectrum of EGFR mutations in patients with NSCLC

Mutation status	Number	%
No mutations detected	108	78.26%
Exon 21 L858R mutation	14	10.14%
Exon 19 deletion mutation	10	7.25%
Exon 20 Ins Mutation	1	0.72%
Unable to perform analysis	5	3.62%

The L858R mutation of exon 21 was more frequent in the considered cohort than the deletion of exon 19 – 56% and 40% of the total number of patients with EGFR mutations, respectively. This is somewhat different from the litera-ture data on cohorts from different countries [8-10] where an exon 19 deletion is most frequent.

Distribution of EGFR mutations by sex in the examined cohort was as follows. Men composed 60.87% of the cohort (n=84) vs. 39.13% of women (n=54). Gene mutations in women (n=12, 22.22%) were more frequent than in men (n=13, 15.48%). The most frequent mutation in both sexes was exon 21s L858R mutation – 9.52% in men and 11.11% in wom-en. The deletion of exon 19 was less frequent – 5.95% in men and 9.26% in women. Also, 1 (1.85%) patient had a rare EGFR 20 Ins mutation (table 3).

Table 3 – Distribution of EGFR mutations in the study cohort by sex

Men (n=84; 60.87%)			Women (n=54; 39.13%)			
Mutation detected (n=13; 15.48%)		No mutation detected (n=71, 84.52%)	Mutation detected (n=12; 22.22%)			No mutation detected (n=42, 77.78%)
L858R	del19ex		L858R	del19ex	Exon20 Ins	
8 (9.52%)	5 (5.95%)		6 (11.11%)	5 (9.26%)	1 (1.85%)	

Conclusions. In its clinical practice, KazIOR utilizes Real-Time PCR to diagnose clinically significant EGFR mutations as they are strong predictors of the response to EGFR-TKI treatment in NSCLC.

78.26% of patients with NSCLC had no EGFR mutations. This indicates a possible presence of other mutations not studied in this spectrum. Further research is needed to determine the exact causes of such results.

EGFR mutations in exons 19, 20 and 21 were found in 15.48% of male and 22.22% of female patients with NSCLC. An exon 19 deletion was detected in 40%, and point replacements of Leu858Arg – in 56% of NSCLC cases.

Diagnostics of gene mutations allows choosing an optimum scheme of treatment based on the tumor drug sensitivity analysis. Thus, the study of the tumor molecular-genetic profile is becoming increasingly important as it allows imple-menting a personalized approach to treatment. It becomes possible with the accumulation of experimental and clinical experience in molecular oncology, better understanding of tumor growth genetic mechanisms, and the introduction of new targeted drugs into clinical practice.

References

1. EGFR gene // ghr.nlm.nih.gov/gene/EGFR#. 14.07.2018;

2. EGFR – epidermal growth factor receptor [Homo sapiens (human)] //www.ncbi.nlm.nih.gov/gene/1956. 14.07.2018;

3. Lopes G. L., Vattimo E.F. de Q., de Castro G. Identifying activating mutations in the EGFR gene: prognostic and therapeutic implications in non-small cell lung cancer // Jornal Brasileiro de Pneumologia. – 2015. – V.41, №4. – P. 365-375;

4. Mishra R., Hanker A.B., Garrett J.T. Genomic alterations of ERBB receptors in cancer: clinical implications // Oncotarget. – 2017. – V.8, № 69. – P.114371-114392;

5. Enikeev R.F., Gordiev M.G., Mommaeva M.S. Testirovanie EGFR-mutatsiy pri rake legkogo. Sobstvennyy opyt [Testing EGFR mutations in lung cancer. Own experience] // Povolzhskiy onkologicheskiy vestnik [Oncology Bulletin of the Volga Region]. – 2012. – №4;

6. Hirsh V. Turning EGFR mutation-positive non-small-cell lung cancer into a chronic disease: optimal sequential therapy with EGFR tyrosine kinase inhibitors // Therapeutic Advances in Medical Oncology. – 2018. –V. 10. – P.1-12;

7. Azzoli C., Baker S., Temin S., Pao W., Aliff T., Brahmer J., Johnson D.H., Laskin J.L., Masters G., Milton D., Nordquist L., Pfister D.G., Piantadosi S., Schiller J.H., Smith R., Smith T.J., Strawn J.R., Trent D., Giaccone G. American society of clinical oncology practice guideline update of chemotherapy for stage IV non-small cell lung cancer // Journal of Clinical Oncology. – 2009. – V.27, № 36. – P. 6251-6266;

8. Kolomeytseva A.A., Gagarin I.M., Mochal'nikova V.V., Mazurenko N.N, Gorbunova V.A. Effektivnost' ingibitorov retseptora epidermal'nogo faktora rosta (EGFR) u bol'nykh rasprostranennym nemelkokletochnym rakom legkogo [Efficacy of epidermal growth factor receptor (EGFR) inhibitors in patients with advanced non-small cell lung cancer] // Vestnik of N.N. Blokhin National Medical Research Center of Oncology. – 2011, №1. –P.42-48;

9. Xie G., Xie F., Wu P., Yuan X., Ma Y., Xu Y., Li L., Xu L., Yang M., Shen L. The mutation rates of EGFR in non-small cell lung cancer and KRAS in colorectal cancer of Chinese patients as detected by pyrosequencing using a novel dispensation order // Journal of Experimental & Clinical Cancer Research. – 2015. – V.34, № 1. – P.63;

10. Mazurenko N.N., TSyganova I.V., Gagarin I.M., Chuev YU.V., Mochal'nikova V.V., Kolomeytseva A.A., Gorbunova V.A. Mutatsii EGFR i KRAS, vazhnye dlya targetnoy terapii nemelkokletochnogo raka legkogo [EGFR and KRAS mutations important for targeted non-small cell lung cancer therapy] // Molekulyarnaya meditsina [Molecular medicine]. – 2013. – №6. – P. 55-59.