**Abstract**

Introduction

As the leading cause of cancer-related deaths, the non-small-cell lung cancer captures the attention of researchers with the desire to design new therapies. Molecular aetiology of non-small-cell lung cancer is related to mutations in epidermal growth factor receptor (EGFR), member of HER/ErbB family of receptors. Non-small-cell lung cancer is not genetically unique, emerging from mutations in exons 19, 20 and 21. Currently, the most advanced and the most widely accepted therapy includes tyrosine kinase inhibitors erlotinib (Tarceva™) and gefitinib (Iressa™). This paper discusses the differential response to targeted therapy in non-small-cell lung carcinoma patients harboring epidermal growth factor receptor mutations.

Conclusion

The crucial and still unsolved question is the examination of treatment response in patients harbouring different mutations. Procedures involve kits that simultaneously detect several mutation types. Through application of spectroscopic and chemometrics, it can be partially solved, but the demand for research kits rather than simple diagnostic kits remains for better understanding of non-small-cell lung cancer.

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**Introduction**

Lung cancer is one of the most common cancers in men and women worldwide. Depending on different aetiologies of the disease, lung cancer can be divided into two major types: small-cell lung cancer (SCLC), which accounts for 20% of lung cancers, and non-small-cell lung cancer (NSCLC), comprising 80% of lung cancers. SCLC is derived from neural crest, while NSCLC originates from lung epithelium. With over one million deaths per year, NSCLC is the leading cause of cancer-related deaths worldwide.

The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase (TK). This cell surface receptor belongs to the HER/ErbB family of receptors, which consist of four distinct receptors: HER1/EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her3 (ErbB-3) and Her4 (ErbB-4). These transmembrane glycoproteins consist of extracellular ligand-binding domain, transmembrane segment and intracellular domain with TK activity. Binding of a ligand to the extracellular domain induces homo- or heterodimerization with another receptor (from the HER family), leading to autophosphorylation of intracellular domain, which is the triggering event for activation of various signalling pathways. Depending on signal transduction mechanism initiated, processes like cancer cell proliferation, migration, angiogenesis and inhibition of programmed cell death are stimulated.

In patients with NSCLC, according to different authors, between 43% and 83% of tumours show some mutation in HER1/EGFR gene. Depending on the population, the frequency of HER1/EGFR point mutations is only 5%–20%. It has been shown that mutations in HER1/EGFR are correlated with poor prognosis and resistance to chemotherapy. However, patients harbouring these mutations tend to show good response to targeted cancer treatments. To use this fact and counteract with the disease progression, novel anticancer agents have been developed, with TK inhibitors (TKIs) being one of the most advanced as specific, targeted therapy for NSCLC.

In this paper, we summarize in brief our current knowledge about the molecular background of NSCLC, with an emphasis on the connection between targeted therapy response and EGFR mutation type. In the last section, we have focused on the drawbacks of modern diagnostic procedures, including the possible ways to overcome them.

**Discussion**

The authors have referenced some of their own studies in this paper. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964), and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

**EGFR mutations classification**

EGFR mutations are usually restricted to NSCLC, with mutations...
occurring at much less frequency in SCLC and some other types of cancer. All documented mutations were found in the intracellular, TK domain of the EGFR. TK domain mutations in EGFR gene belong to specific types of mutations that confer sensitivity to EGFR TKIs. These mutations are often called 'activating mutations', since they lead to ligand-independent activation of TK activity and to the promotion of oncogenic events. They are usually divided into three categories: (i) in-frame deletions in exon 19, (ii) single-nucleotide substitutions and (iii) in-frame duplications and insertions in exon 20. The vast majority of activating mutations fall into class (i) and (ii).

Class (i) mutations in exon 19 mostly include deletions of amino acid residues leucine to glutamic acid (ΔLRE), and they represent the majority of EGFR-TK activating mutations. In class (ii), the most prevalent type of mutation is an exon 21 substitution of arginine for leucine at codon 858 (L858R). In this class, mutations glycine-719 (G719) to serine, alanine or cysteine are also included, as well as some other missense mutations.

Differential response to EGFR-targeted therapy

Activation EGFR mutations largely involve the adenosine triphosphate (ATP)-binding pocket in the TK domain of the receptor (Figure 2). Orally administered TKIs with their low molecular weight compete with ATP for the binding site in the ATP-binding pocket, thus preventing the receptor’s catalytic activity and autophosphorylation of the intracellular domain. In this way, activation of the downstream signalling pathway is inhibited.

The most advanced and widely used TKIs are erlotinib (Tarceva®) and gefitinib (Iressa®). These reversible, ATP-competitive EGFR TKIs show significant clinical response in 10%–30% of NSCLC patients, depending on smoking history, ethnic origin and sex. Many studies indicated correlation between the presence of activating mutations and positive response to erlotinib and gefitinib. Moreover, it is believed that these mutations represent very early genetic events in the lung cancer development. However, there are significant differences between EGFR-TK-activating mutations in response to small-molecule inhibitors. NSCLC patients with exon 19 deletion (ΔLRE) treated with erlotinib or gefitinib survive longer than those with L858R mutation. Similarly, cancer cells expressing the L858R mutant show higher sensitivity to gefitinib than those expressing G719S mutant. Overall, in patients with exon 19 mutations, response rates to EGFR TKIs are significantly higher (70%–100%) than that in patients with exon 21 mutations (20%–67%).

Despite very promising statistics regarding EGFR TKIs response rates, some lung carcinomas fail to respond to this type of therapy, which is considered to be primary resistance to treatment. Moreover, in the vast majority of patients with...
Initially good therapy response, disease progression has been noted over time. This type of resistance is called acquired (secondary) resistance to therapy. Primary lack of response to EGFR TKIs is in most cases consequence of additional genetic alteration. These alterations include exon 20 insertions D770_N771 (ins NPG), D770_N771 (ins SVQ) and D770_N771 (ins G), N771T. It should be noted that exon 20 insertions are relatively rare, which leads to the conclusion that there are other, still unknown, mechanisms involved in primary resistance to therapy.

In more than half of all patients, drug efficacy is altered by a second point mutation in the TK domain. It is believed that this emergence of acquired resistance is due to selective pressure during treatment, as it is rarely detected in untreated patients. In the EGFR gene, there are two documented resistance point mutations to the TKIs gefitinib and erlotinib, exon 20 mutation threonine-790 to methionine (T790M), and exon 19 mutation aspartic acid-761 to tyrosine (D761Y). Acquisition of T790M, which is also called gatekeeper mutation, leads to distortion in the ATP-binding pocket, altering ATP affinity of the TK domain of the EGFR gene. D761Y mutations are believed to weaken interaction of EGFR TKI with its target, which leads to ineffectiveness of targeted therapy.

Diagnostic procedures and possible optimization
It was already mentioned that activating mutations show differential response to targeted therapy with TKIs. From this fact, the assumption arose—is it possible to obtain different treatment responses from different activating mutations that belong to the same type? This is confirmed by recent findings, which indicated that different deletion types in the 19th exon of the EGFR gene have shown different in vitro sensitivities to the tyrosine kinase inhibitors.

In NSCLC, exon 19 deletions are the most prevalent somatic mutations. Deletion detection is an indication for targeted therapy with TKIs in patients with NSCLC. In general, patient harbouring any type of deletion in exon 19 of EGFR gene is considered a candidate for this type of treatment. According to the widely accepted hypothesis that all known ΔLRE mutations express the same phenotypic effect in terms of response to targeted therapy, commercially available EGFR mutation detection kits allow detection of the aforementioned deletions, but do not distinguish between them. Modest precision of existing diagnostic procedures and insufficient information regarding the exact mutation type could be overcome by using relatively simple mathematical approach developed by Jovanović et al. This method is based on the basic physical property of fluorescence probes used for detection of real-time PCR products. If the fluorophore is presented to different microenvironments originating from different DNA sequences, spectral properties (overall shape and maxima position) could be affected. In spectrofluorimetry, such property is exploited for the detection of conformational changes in molecules such as proteins.

Conclusion
During the past years, knowledge about molecular mechanisms causing NSCLC has accumulated enough for the development of target treatments. As the majority of NSCLC is caused by mutations in EGFR, target therapy is concentrated to tyrosine kinase inhibitors. However, some major issues remain. Current procedures used for the detection of EGFR mutations are based on commercial real-time PCR kits. Diagnostic procedures are fast, but potentially valuable information about therapy response in patients with different mutations remains unknown. Synergistic effects of several mutations occurring simultaneously also remain unexplained. Reported data show significant variations leading to the conclusion that improved accuracy of diagnostic methods is the foundation for successive therapy.

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References
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