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Determination of *EGFR* Gene Somatic Mutations in Tissues and Plasma of Patients with Non-Small Cell Lung Cancer

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Abstract—Activating mutations in the *EGFR* gene influence cell proliferation, angiogenesis, and increases metastatic ability of non-small cell lung cancer (NSCLC) cells; they have a significant impact on the choice of medical therapy of NSCLC. The use of targeted therapy with tyrosine kinase inhibitors requires performance of appropriate genetic tests in NSCLC patients. The aim of this study was to develop a real-time PCR-based diagnostic test-system for rapid and cost-effective analysis of *EGFR* mutations in paraffin blocks and plasma and to perform comparative estimation of diagnostic characteristics features of real-time wild type blocking PCR and digital PCR. The study included 156 patients with different degrees of lung adenocarcinoma differentiation. A simple and efficient real-time PCR-based method for detection of *L858R* activating mutation and *del19* deletion in the *EGFR* gene in DNA isolated from paraffin blocks or blood has been developed. The test system for *EGFR* mutations has been validated using 411 samples of paraffin blocks. The proposed system demonstrated high efficiency for DNA testing from paraffin blocks: a concordance with results of testing by means a Therascreen® EGFR RGQ PCR Kit (Qiagen, Germany) was 100%. Applicability of this test system has been also demonstrated for detection of mutations in plasma.

Keywords: EGFR gene, activating mutations, real-time PCR, non-small cell lung cancer, plasma
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INTRODUCTION

The *EGFR* gene encodes the epidermal growth factor receptor (EGFR), a transmembrane glycoprotein, a member of the receptor tyrosine kinase family. EGFR influences angiogenesis, proliferation, and increases metastatic activity of cells [1, 2]. The presence of mutations in the *EGFR* gene results in receptor dimerization, autophosphorylation of its intracellular tyrosine kinase domain and activation of MAPK and PI3K/Akt signaling pathways [3]. Phosphorylated proteins, in turn, activate transcription factors that regulate synthesis of mRNA and proteins [4]. Most of the somatic mutations of the *EGFR* gene found in patients with non-small cell lung cancer (NSCLC) are localized in the exons 18–21 encoding the tyrosine kinase domain [5, 6]. In this context mutations associated with susceptibility to tyrosine kinase inhibitors (ITK) are especially interesting. These mutations are: deletions in exon 19 (del19), which may be of different sizes [3], *L858R* replacement in exon 21 [7, 8], and also mutations associated with resistance to ITK, for example, the *T790M* mutation in exon 20 [7–9].

One of the first methods used for determining somatic mutations in the *EGFR* gene was Sanger sequencing, which involved analysis of the whole coding region [10]. However, this method has a low sensitivity (about 20% of the mutant DNA content in the sample), so more sensitive methods based on direct analysis of mutations and deletions of the *EGFR* gene have been developed [11, 12]. Current methods for the analysis of somatic mutations represent either targeted gene sequencing, or whole genome/whole-exome sequencing [13, 14]. However, these methods are expensive, and existing test systems of Russian manufacturers are not adapted for routine use in clinical diagnostic laboratories.

It should be also noted modern molecular genetic studies use mostly DNA isolated directly from the tumor tissue. The surgical material is usually available for analysis, but detection of mutations in biopsy