DETECTION OF EGFR T790M AND L858R MUTATIONS BY SEQUENCING OR QUANTITATIVE REAL-TIME PCR METHOD: THE SENSITIVITY COMPARISON

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Incidence of EGFR tyrosine kinase domain activating mutations is a qualifying criteria for tyrosine kinase inhibitors' (TKI) therapy in non-small cell lung cancer (NSCLC) patients. 90% of diagnosed EGFR mutations comprise microdeletions in exon 19 and point missense mutation L858R in exon 21. Exon 20 T790M mutation encodes resistance to reversible TKI's. Importantly, most NSCLS tissue samples presented for molecular analysis are genetically heterogenic with respect to the EGFR mutations. Although direct sequencing is the common strategy to determine these alterations, its threshold point for mutant allele detection in population of wild type DNA is according to literature close to 10%. Meanwhile, it is assumed that probe-based methods are more sensitive in comparison to sequencing. The aim of this study was to compare the detection threshold for EGFR T790M and L858R mutations by quantitative real-time PCR with allele-specyfic TaqMan probes and direct sequencing methods. PCR analysis was performed with dual-labelled fluorescent probes complementary for one of the specific mutation sites or for wild-type allele. DNA isolated from H1975 cell line, heterozygous for T790M and L858R mutations, was mixed with control wild-type DNA from peripheral blood lymphocytes in 1:1, 1:4, 1:9, 1:19, 1:49, 1:99 ratio, that comprised 25% to 0,5% mutant allele to wild type ratio, respectively. Demonstrated detection sensitivity for T790M and L858R mutation was as low as 2,5% of mutant allele in the wild-type background for TaqMan probes while up to 5% for direct sequencing. Fluorescent probe-based quantitative real-time PCR provided specific and sensitive detection of analyzed EGFR mutations, despite the considerable background of wild type allele. This method proved diagnostically useful, fast, cost-effective and at least twice more sensitive than sequencing.