Clinical Evaluation of An Improved Method for Epidermal Growth Factor Receptor Mutation Detection

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[Abstract]

Background: Since targeting the epidermal growth factor receptor(EGFR) is an attractive strategy for the treatment of advanced non-small cell lung cancer(NSCLC), the detection of somatic EGFR mutations plays an important role in the therapeutic decision making. Direct sequencing is an attractive method for detecting both known and unknown mutations, and therefore has become one of the most widely used methods in EGFR mutation detection and considered as the gold standard. But its drawbacks are time consuming , sensitivity limited and tending to contamination of non-malignant cells in samples. Another most widely used method is the Amplification Refractory Mutation System (ARMS), a highly sensitive and fast method in point mutation detection. However, We have achieved several improvements in the testing procedure of Fluorescence Polymerase Chain Reaction(PCR), and established a highly sensitive, easy, rapid and inexpensive assay. The detection sensitivity is 0.1% of mutant DNA in the presence of its wild-type DNA.

Hypothesis: The aims of this research were to evaluate the assay' s ability to detect EGFR mutations, and compare the sensitivity, specificity and accuracy to direct sequencing and amplification refractory mutation system (ARMS).

Methods: From June 2013 to August 2015, one hundred and forty-one FFPE samples of resected NSCLC were collected in Hunan Cancer Hospital. Direct sequencing, amplification refractory mutation system (fluorescence PCR) and the novel assay were used to detect EGFR mutations separately, and the operators were blind to the results(single blind) at the same time. The differences of these methods were then further analyzed.

Results: The detection success rates of all three methods were 100%. The consistent rate (completely same points and same mutation types) of the novel assay and direct sequencing

was 75.9%(107/141). Among 96 samples with EGFR mutations detected via direct sequencing, the same mutations were detected in 92 samples via the novel assay(95.8%). However, in the other 45 samples without mutations tested by direct sequencing, 23 samples(51.1%) were found to be EGFR mutation-positive using the novel assay, and there were significant differences between these two methods ($X2 = 40.745$, $P < 0.05$) . Compared with direct sequencing as the gold standard, the sensitivity, specificity of the novel assay were 95.8%, 48.9%, respectively. The positive predictive value and negative predictive value were 80.0%, and 84.6%, respectively, with the accuracy of 80.9%. When compared with the results of ARMS, the consistent rate of these two methods reached 84.4%(119/141), with a high consistence (Kappa = 0.749, $P < 0.05$).