Mutation-Specific Antibodies for the Detection of EGFR Mutations in Non-Small-Cell Lung Cancer

Summary
Activating mutations within the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) are found in approximately 15-20% of non-small-cell lung cancer (NSCLC) patients and are associated with improved response to the EGFR inhibitor Gefitinib and Erlotinib. The most common NSCLC-associated EGFR mutations are the exon 19 deletions (E746-A750del) and the exon 21 point mutation (L858R). Together these mutations account for 95% of EGFR mutations. Here, we generate mutation-specific rabbit monoclonal antibodies against each of these EGFR mutations. The mutation-specific antibodies detect the corresponding mutant form of EGFR but not wild type EGFR by Western blotting, immunofluorescence (IF), and immunohistochemistry (IHC). IHC screening of a large panel of paraffin-embedded NSCLC tumor samples demonstrates these antibodies are highly sensitive and specific. This IHC assay provides a rapid, sensitive, specific and cost-effective method to identify lung cancer patients likely to respond to EGFR-targeted therapies.

Introduction
The most common NSCLC-associated EGFR mutations, the exons 19 deletion (E746-A750del) and the exon 21 substitution (L858R), account for 95-96% of EGFR mutations. The remaining mutations occur at much lower frequencies including those in exon-20 and codon 719.

Direct DNA sequencing to detect EGFR mutations in patient tumor tissue is currently the standard method. However, acceptance of this technique has been hindered by the high cost of equipment and reagents, technical difficulties of performing the assay and length of the procedure. While other DNA-based methods have been developed, these methods are not routine procedures in clinical labs and require expensive and time-consuming processing.

In contrast, immunohistochemistry (IHC) is a well-established method routinely used in solid tumor diagnosis in clinical laboratories. IHC also allows for the analysis of small tissue samples or individual cells obtained from a variety of sources including circulating tumor cells. Thus, the development of antibodies that specifically detect mutant EGFR protein by IHC would be a valuable addition to the current protocols utilized in the diagnosis and treatment of lung cancer.

Materials and Methods
Human NSCLC Tumor Tissues: FFPE- or paraffin-embedded samples were provided by the Second Xiangya Hospital, Central South University (Changsha, Hunan, P.R.China). Human NSCLC tumor samples demonstrate these antibodies are highly sensitive and specific. This IHC assay provides a rapid, sensitive, specific and cost-effective method to identify lung cancer patients likely to respond to EGFR-targeted therapies.

Conclusions
Recent clinical results suggest that the treatment of NSCLC patients with EGFR-targeted therapies based on the status of EGFR mutations can improve patient response. To address the need to characterize the mutational status of patients, we generated two mutation-specific rabbit monoclonal antibodies that specifically detect the two most common EGFR mutations in NSCLC. We showed that these antibodies are highly sensitive and specific and can be used in conventional IHC assays to identify tumors harboring these EGFR mutations. Importantly, this assay maintains tumor morphology, allows tumor heterogeneity to be studied and eliminates the false negatives seen in DNA sequencing methods due to an inadequate percentage of tumor cells.

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