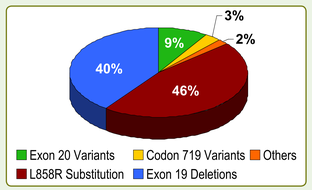
Mutation-Specific Antibodies for the Detection of EGFR Mutations in Non-Small-Cell Lung Cancer

Summary

Activating mutations within the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) are found in approximately 10-20% of non-small cell lung cancer (NSCLC) patients and are associated with improved response to the EGFR inhibitors Gefitinib and Erlotinib. The most common NSCLC-associated EGFR mutations are the exon 19 deletion (E746-A750del) and the exon 21 point mutation (L858R). Together these mutations account for 90% 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 exon 19 deletion (E746_A750del) and the exon 21 substitution (L858R) account for 85-90% of EGFR mutations. The remaining mutations occur at much lower frequencies including those in exon-20 and codon 719.



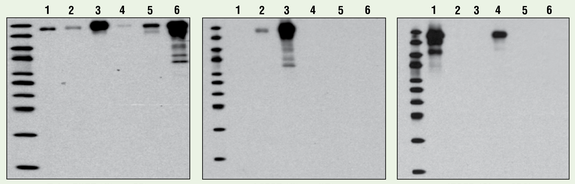
Direct DNA sequencing to detect EGFR mutations in patient tumor tissue is currently the standard method. However, acceptance of this technique has been hindered by the high cost of equipment and reagents, technical difficulties of performing the assay and length of the procedure. While other DNA-based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

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

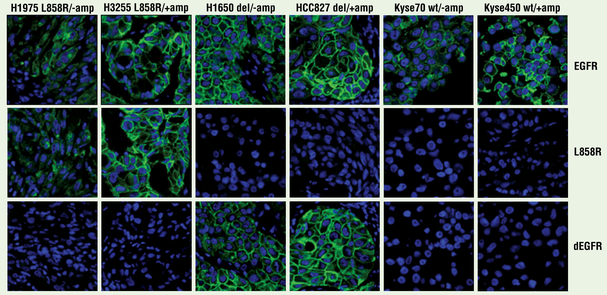
Human NSCLC Tumor Tissues: IRB approval was granted by the Second Xiangya Hospital, Central South University (Changsha, Hunan, P.R.China). Human samples of NSCLC paraffin blocks were provided by the Second Xiangya hospital.

Western blot analysis of whole cell lysates from cancer cell lines with control EGFR antibody, L858R specific antibody and dEGFR specific antibody.

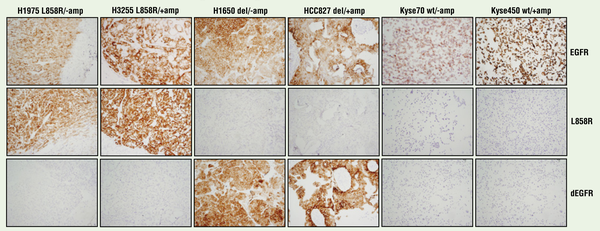


1. HCC827 (dEGFR) **2.** H1975 (L858R) **3.** H3255 (L858R) **4.** H1650 (dEGFR) **5.** Kyse70 (wtEGFR) **6.** Kyse450 (wtEGFR)

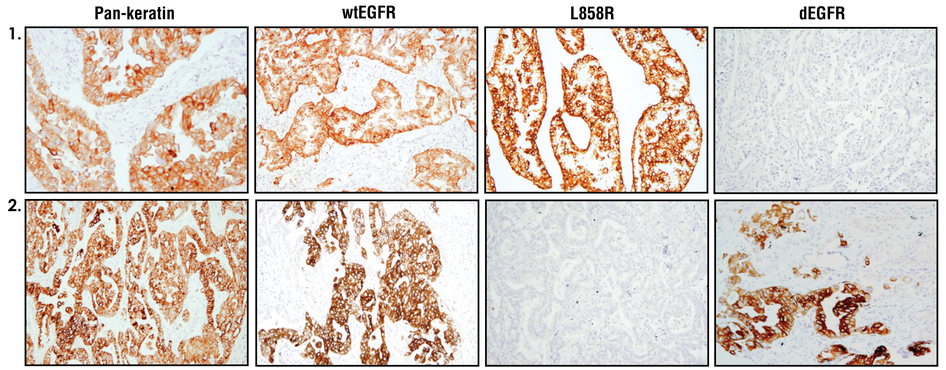
Immunofluorescence (IF) staining of xenografts.



Immunohistochemistry (IHC) staining of xenografts.



Immunohistochemical staining of two NSCLC tumor samples.



IHC results of NSCLC tumor samples.

Pathology	Number	L858R (+)	dEGFR (+)
AC	217	28	23
SCC	112	0	1
LCC	11	0	0
Total	340	28	24

Comparison of IHC results and Direct DNA sequencing analysis.

A. L858R Mutation

IHC	DNA Sequencing		
	L858R	wt	failed
L858R (+)	24	2	2
L858R (-)	2	193	25

B. Exon 19 Deletion

IHC	DNA Sequencing		
	dEGFR	wt	failed
dEGFR (+)	23	0	1
dEGFR (-)	3	196	23

Comparison of MS Sequencing, Direct Sequencing and IHC.

	Exon 19 Deletion	L858R	
	MS	MS	failed
Direct DNA Sequencing: Incorrect / Total	5 / 9	3 / 9	2 / 18
IHC: Incorrect / Total	2 / 9	0 / 9	0 / 18

Sensitivity: 92% **Specificity: 99%**

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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