IHC with Mutation-Specific Antibodies Detecting the Status of EGFR Mutations in NSCLC

Jian Yu[1], Daiqiang Li[2], Chunhong Hu[3] • Cell Signaling Technology[1] Danvers, MA, USA • Department of Pathology[2], Department of Oncology[3], Second Xiangya Hospital, Changsha, China

Background:

Activating mutations within the tyrosine kinase of EGFR are found in approximately 10-30% of NSCLC patients.

The association between EGFR mutations and response to EGFR TKIs in NSCLC has been consistently confirmed in a number of studies.

Deletions in Exon 19 and the L858R substitution in Exon 21, accounting for approximately 90% of all EGFR mutations, are the best characterized sensitizing mutations in AC.

IHC is available to all pathology department and can be performed on the samples with small numbers or small proportion of tumor cells.

This study was designed to evaluate IHC with mutation specific antibodies to detect mutant EGFR proteins in NSCLC for patient selection to TKI therapy.

Materials and Methods:

430 formalin-fixed, paraffin-embedded NSCLC tumor samples (307 AC, 112 SCC and 11 LCC) from Second Xiangya Hospital were used under IRB-approved protocols.

IHC was performed on whole tissue section using EGFR mutation specific antibodies (from Cell Signaling Technology®).

DNA was extracted from the samples and direct DNA sequencing was performed to confirm the IHC result. Selected cases were confirmed by higher sensitive DNA sequencing (Mass-Spectrometry).

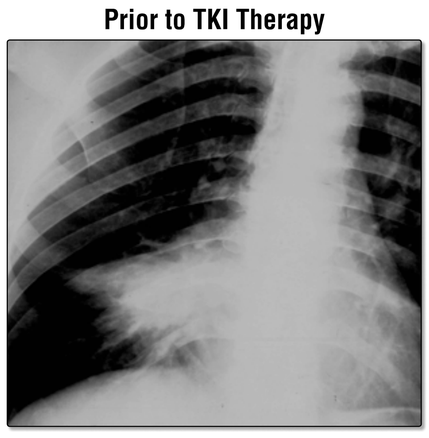
Clinical data was collected from 32 cases of the patients treated by TKI prior the screening.

Results:

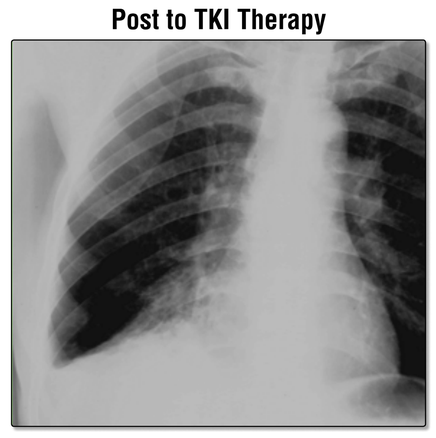
IHC Result of NSCLC Tumor Samples.

Pathology	Number	L858R (+)	dEGFR (+)
AC	307	37	33
SCC	112	0	1
LCC	11	0	0
Total	**430**	**37**	**34**

Prior to TKI Therapy



Post to TKI Therapy



Comparison of IHC and DNA Sequencing Results in Exon 19 Deletion and L858R:

IHC and Molecular Assay Results in L858R.

IHC Seq.	L858R (+)	L858R (-)	Total
IHC (+)	33	2	35
IHC (-)	3	392	395
Total	**36**	**394**	**430**

Sensitivity: 91.6% (33/36) • **Specificity: 99%** (392/394)

IHC and Molecular Assay Results in EGFR Exon 19 Deletion.

IHC Seq.	Exon 19 (+)	Exon 19 (-)	Total
IHC (+)	33	0	33
IHC (-)	7	390	397
Total	**40**	**390**	**430**

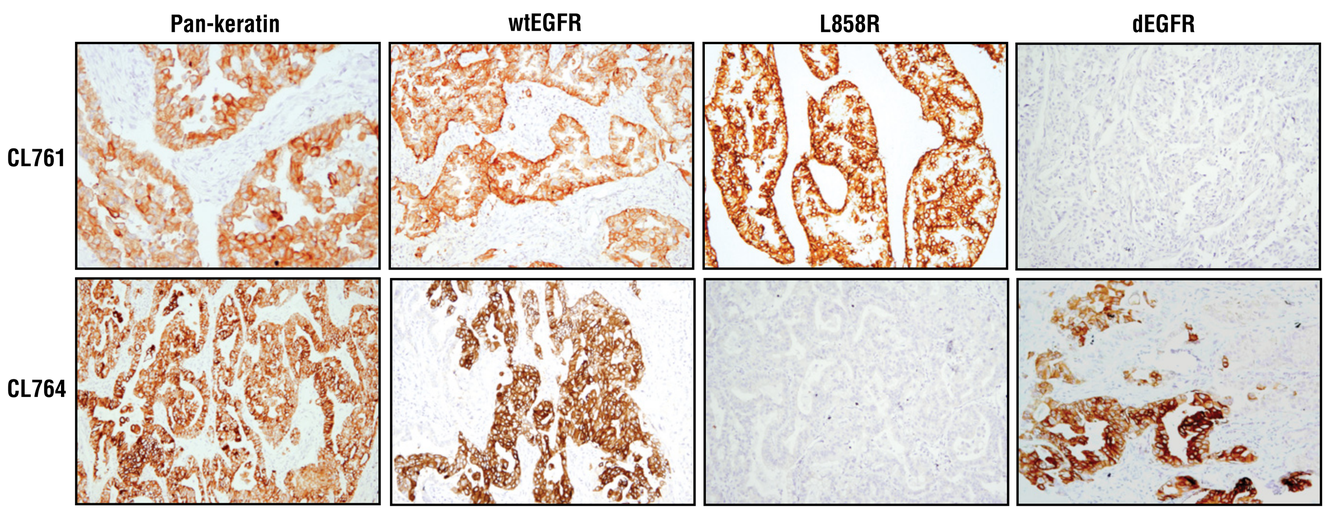
Sensitivity: 82.5% (33/40) • **Specificity: 100%** (390/390)

Types of Exon 19 Deletion and IHC Results.

IHC Seq.	E746_A750del	Others	Total
IHC (+)	31	2	33
IHC (-)	3	4	7
Total	**34**	**6**	**40**

Sensitivity: 91% (31/34)
Other Rare Types of Exon 19 Deletion: 33.3%

Examples of IHC Staining with a Panel of Four Antibodies:



Comparisons between clinical and IHC Selection in 32 advanced cases treated by Iressa for three months.

Outcomes	Clinical Selection	IHC Staining (+)
Complete Response (CR)	1	1
Partial Response (PR)	8	7
Stable Disease (SD)	2	1
Progressive Disease (PD)	21	0
Total	**32**	**0**
Total Effect Rate (CR+PR)	**28.1%**	**88.9%**
Disease Control Rate (CR+PR+ SD)	**34.4%**	**100%**

IHC Detecting Either of the EGFR Mutations: Sensitivity: 87% • Specificity: 99%

Conclusion:

IHC with mutation specific antibodies to detect mutant EGFR proteins is a rapid, specific and cost efficient Methodology.

The assay preserves the morphology of the cells and it can be used to identify the mutations from the samples with small number or small portion of tumor cells.

The positive staining is not just highly correlative to the sequencing result, but also correlative to tumor response to TKIs.

References:

Yu, J., Kane, S., Wu, J. et al. (2009) Mutation-specific antibodies for the detection of EGFR mutations in non-small-cell lung cancer. *Clin Cancer Res.* 15, 3023–3028.

Brevet, M., Arcila, M. and Ladanyi, M. (2010) Assessment of EGFR Mutation Status in Lung Adenocarcinoma by Immunohistochemistry Using Antibodies Specific to the Two Major Forms of Mutant EGFR. *J Mol Diagn.* 12, 169–176.

Jian Yu • Cell Signaling Technology • Email: jyu@cellsignal.com