Immunohistochemical Analysis of Potential Cell Signaling Biomarkers for Mutant Epidermal Growth Factor Receptor.

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Background

Recent studies have shown that mutations in the Epidermal Growth Factor Receptor (EGFR) may be responsible for some patient sensitivity to EGFR kinase inhibitors such as Iressa and Tarceva; therefore, potential cell signaling biomarkers for mutant EGFR would be clinically useful.

Design

Using immunohistochemical (IHC) and tissue microarray (TMA) techniques, we studied EGFR phosphorylation and its downstream molecules in five human Non-small Cell Lung Cancer (NSCLC) xenograft models and 228 NSCLC patient samples and compared EGFR and Akt pathway activation with EGFR mutation status. Slides were immunostained with activation-state specific antibodies, including antibodies to phospho-EGFR, phospho-tyrosine and phospho-Akt. 12 cases were subjected to EGFR DNA sequencing.

Results

- High EGFR phosphorylation of all the tyrosine sites probed and phosphorylated Akt were detected in mutant EGFR cell xenografts but not in xenografts with wild type EGFR.

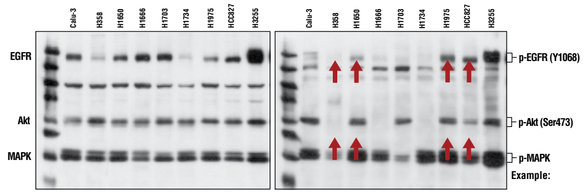
- IHC analysis of general phospho-tyrosine, phospho-EGFR and phospho-Akt on a NSCLC TMA found phosphorylated EGFR in 15%-25% of patient samples, depending on the tyrosine site. Phosphorylated EGFR was more frequent in adenocarcinomas (20%-30%) and in bron choalveolar carcinomas (BAC) (28%-50%) than in squamous cell carcinomas (SCC) (5%-10%).

- The phospho-EGFR reactivity was closely correlated with high general phospho-tyrosine reactivity. There was also a strong correlation between high phospho-EGFR staining and high phospho-Akt staining in patient tissue samples.

- EGFR kinase domain DNA sequencing revealed that a majority of these samples with high EGFR and Akt phosphorylation were mutant.

EGFR and Akt are constitutively phosphorylated in EGFR mutant NSCLC cell lines and Xenograft tissues.

Cell Lines: Western Blots



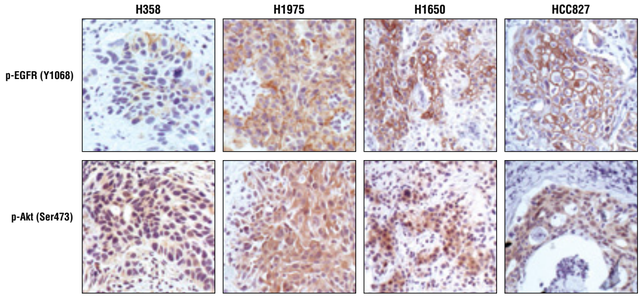
Cell Line Information

Cell line	Cell ID	EGFR Mutation	Ras
H358	BAC	wt	mut
H1650	BAC	DelE746A750	wt
H1975	AC	L858R, T790M	wt
HCC827	AC	Del722-726	wt

WB analysis of various NSCLC cell lines demonstrating high EGFR and Akt phosphorylation in EGFR mutant cell lines (H1975, H1650, HCC827), but not EGFR wild type cell line (H358).

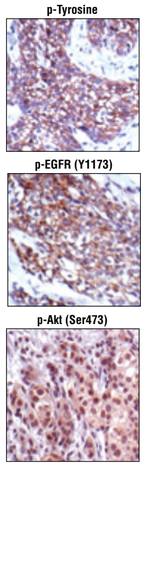
Xenograft Tissues: IHC Staining

	H358	H1975	H1650	HCC827
EGFR	1, cyt	2-3, cyt/mem	2-3, cyt/some	3, cyt/mem
p-EGFR(845)	1, mem/cyt	3, mem/cyt	3, mem/cyt	3, mem/some cyt
p-EGFR(Y992)	0-1, cyt	3, mem/cyt	3, mem/cyt	3, mem/some cyt
p-EGFR(Y1045)	0	1-2, cyt	0-1, cyt	2-1, mem/some cyt
p-EGFR(Y1068)	**0-1, mem/cyt**	**3, mem/cyt**	**3, mem/cyt**	**3, mem/cyt**
p-EGFR(Y1148)	0	0	1, mem/cyt	2, mem/cyt
p-EGFR(Y1173)	2, cyt/mem	3, mem/cyt	3, mem/some cyt	3, mem/some cyt
p-Akt (Ser473)	**0-1, cyt**	**2-3, cyt**	**2-3, cyt/nuc**	**2, nuc/cyt**
p-Erk	1, nuc	2-1, nuct	2-3, nuc/cyt	2-3, nuc/cyt



IHC analysis of Xenograft tissues demonstrating high levels of EGFR phosphorylation oat all major tyrosine sites in EGFR mutant tissues (H1975, H1650, HCC827), but not EGFR wild type tissue (H358).

EGFR phosphorylation and association with general Tyrosine/Akt phosphorylation in NSCLC patient tissues.



		p-EGFR Positive							p-EGFR Negative							
CST Name	I D	p-Tyrosine	p-EGFR (Y992)	p-EGFR (Y1045)	p-EGFR (Y1173)	p-EGFR (Y1068)	p-EGFR (1148)	p-Akt (Ser473)	CST Name	I D	p-Tyrosine	p-EGFR (Y992)	p-EGFR (Y1045)	p-EGFR (Y1173)	p-EGFR (Y1068)	p-EGFR (1148)	p-Akt (Ser473)
Lung 7	AC, grade 1	cyt/mem, 3	cyt/mem, 3	cyt, 2	cyt, 3	cyt, 2	cyt, 3	2	**Lung 3**	SCC, grade 1	cyt, 1						1
Lung 98	carcioid	cyt, 3	cyt, 3	cyt, 1	cyt, 3	cyt, 1	cyt, 1	3	**Lung 36**	SCC, grade 1-2	cyt, 1						1
Lung 81	BAC, grade 1	cyt, 2	cyt/mem, 3	cyt, 3	cyt, 3	cyt, 3			**Lung 62**	AC, grade 2	cyt, 1						
Lung 79	BAC, grade 1	cyt,1	cyt,1	cyt, 2	cyt, 2	cyt, 2	cyt, 2	3	**Lung 64**	AC, grade 3	cyt, 1						
Lung 63	AC, grade 3	cyt/mem, 3	cyt/mem, 3		cyt/mem, 3			2	**Lung 43**	SCC, grade 2-3	cyt, 2						2
Lung 66	AC, grade 1/ 3	cyt/mem, 2	cyt, 2		cyt,1		mem, 1	2	**Lung 26**	SCC, grade 3	cyt, 2						3
Lung 73	BAC, grade 1	cyt, 3	cyt,1	cyt,2	cyt,1	cyt, 2		3	**Lung 27**	SCC, grade 2	cyt, 1						
Lung 38	SCC, grade 3	cyt/mem, 3	cyt/mem, 2		cyt/mem, 3	cyt, 1			**Lung 28**	SCC, grade 2-3	cyt, 1						
Lung 5	SCC, grade 3	cyt/mem, 3	cyt, 1		cyt, 1			3	**Lung 30**	SCC, grade 2-3	cyt, 1						
Lung 14	carcioid	cyt, 3			cyt, 1	cyt, 2		3	**Lung 31**	SCC, grade2-3	cyt, 2						2
Lung 94	carcioid	cyt, 2		cyt,1		cyt, 1			**Lung 15**	AC, grade 1	cyt, 1						
Lung 58	AC, grade 1-2	cyt/mem, 2			cyt, 1			2	**Lung 23**	SCC, grade 1	cyt, 1						
Lung 77	BAC, grade 1	cyt, 2	cyt, 1	cyt, 3	cyt, 2	cyt, 3			**Lung 24**	SCC, grade 2	cyt, 2						3
Lung 75	BAC, grade 1	cyt, 3		cyt, 2	cyt, 2	cyt, 3		3	**Lung 25**	SCC, grade 2-3	cyt/mem, 2						
Lung 91	carcioid	cyt, 3	cyt, 3	cyt, 3				3	**Lung 67**	AC, grade 1	cyt, 1						
Lung 52	AC, grade 3	cyt, 1		cyt, 1	cyt, 1	cyt, 1		2	**Lung 71**	AC, grade 2	cyt, 1						
Lung 80	BAC, grade 1	cyt, 3				cyt, 3			**Lung 72**	AC, grade 3	cyt, 1						

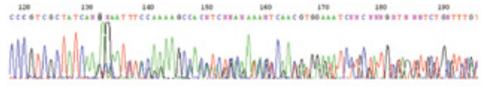
IHC analysis of human patient samples demonstrating a closely correlation of p-EGFR and general p-tyrosine or p-Akt staining.

Comparison of p-EGFR and p-Akt IHC staining of human patient samples with EGFR mutation status.

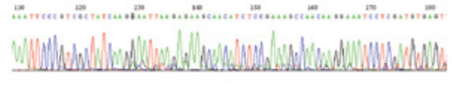
		IHC staining		EGFR mutation	
slide label	Cancer type	p-EGFR*	p-Akt	exon 19	exon 21
lung 98	Carcioid	3+, cyt	3+	del 746-750	WT
lung 63	Adenocarcinoma	3+, cyt/mem	2+	del 746-750	WT
lung 66	Adenocarcinoma	2+, cyt	2+	del 746-750	WT
HL 5	Adenocarcinoma	2+, cyt	1+	point mutation? R	WT
lung 7	Adenocarcinoma	3+, cyt/mem	2+	WT	WT
HL 20	BAC	3+, cyt	0	del 746-750	WT
lung 81	BAC	3+, cyt/mem	0	WT	L858R
lung 38	Sequmous cell carcinoma	3+, cyt/mem	0	WT	WT
lung 80	BAC	3+, cyt	0	WT	contaminated
lung 67	Adenocarcinoma	-	0	contaminated	WT
lung 57	Adenocarcinoma	-	0	WT	WT
HCC 827	Adenocarcinoma	3+, mem/cyt	2+	Homo del 746-750	WT

**at least positive for one phospho-site of EGFR.*

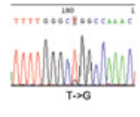
Lung 98, exon 19, del 746-750



Lung 63, exon 19, del 746-750



Lung 81, exon 21, L858R



Conclusion

These results suggest that mutant EGFR is constitutively phosphorylated and activates downstream Akt in NSCLC; therefore, IHC analysis of phosphorylated EGFR and Akt may reflect the activation of mutant EGFR signaling in patient samples.

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