Immunoafinity Profiling of Tyrosine Phosphorylation in Lung Cancer

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Introduction

Tyrosine kinases play a prominent role in human cancer, yet the oncogenic signaling pathways driving cell proliferation and survival have been difficult to identify, in part because of the complexity of the pathways and in part due to low cellular levels of tyrosine phosphorylation. In general, global phosphoproteomic approaches reveal small numbers of peptides containing phospho-tyrosine. We have developed a strategy **PhosphoScan™** that emphasizes the phospho-tyrosine component of the phosphoproteome and identifies large numbers of tyrosine phosphorylation sites (Rush, J. et al. 2004 *Nature Biotech.* 23, 94–101). Peptides containing phospho-tyrosine are isolated directly from protease-digested cellular protein extracts with a phospho-tyrosine specific antibody and are identified by tandem mass spectrometry. We have applied this approach to over lung cancer cell lines and tumor samples, and shown it can be used to identify activated protein kinases and downstream proteins without prior knowledge of the signaling networks that are activated, a first step in profiling normal and oncogenic signaling networks. When coupled to quantitative methods our technology can help identify phosphorylation signatures of drug response.

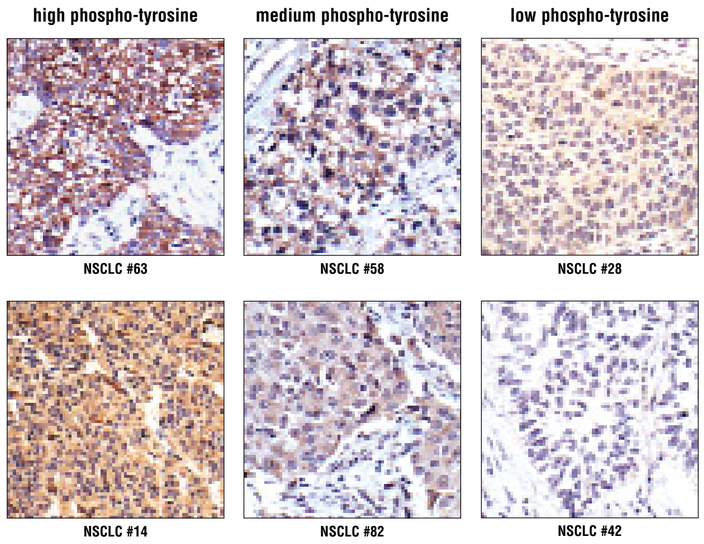

Figure 1.
Representative phospho-tyrosine staining of human lung cancer tissue which can be classified as high, medium, and low phospho-tyrosine signaling.

				p-EGFR					p-HER2			Other p-RTKs			
NO	CST Name	ID	9411, p-Tyrosine	2235, p-EGFR (Y992)	2237, P-EGFR (Y1045)	Y1173, P-EGFR (Y1173)	2236, P-EGFR (Y1068)	4404, p-EGFR (1148)	2245, P-HER2 (Y1112)	2244, P-HER2 (Y1248)	2249, P-HER2 (Y1221/2)	3151, P-M-CSFR (Y723)	3126, P-Met (Y1234/5)	3161, p-PDGFR (Y751)	3391, p-c-Kit (Y719)
1	Lung 7	AC, grade 1	cyt/mem, 3	cyt/mem, 3	cyt, 2	cyt, 3	cyt, 2	cyt, 3	cyt, 1	cyt, 1	cyt, 3	cyt, 3	cyt/mem, 3	cyt, 2	cyt/mem, 3
2	Lung 98	carcioid	cyt, 3	cyt, 3	cyt, 1	cyt, 3	cyt, 1	cyt, 1	cyt, 1	cyt, 1		cyt, 3	cyt, 3	cyt, 3	cyt, 3
3	Lung 81	BAC, grade 1	cyt, 2	cyt/mem, 3	cyt, 3	cyt, 3	cyt, 3		cyt, 3	cyt, 2		cyt, 1	cyt/mem, 2	cyt, 1	cyt, 2
4	Lung 79	BAC, grade 1	cyt, 1	cyt, 1	cyt, 2	cyt, 2	cyt, 2	cyt, 2	cyt, 3	cyt, 1	cyt, 1				cyt, 1
5	Lung 63	AC, grade 3	cyt/mem, 3	cyt/mem, 3		cyt/mem, 3			cyt, 2		cyt/mem, 1	cyt/mem, 3	cyt/mem, 3	cyt/mem, 3	cyt/mem, 2
6	Lung 66	AC, grade 1/3	cyt/mem, 2	cyt, 2		cyt, 1		mem, 1	cyt, 1		cyt/mem, 2		cyt/mem, 1		
7	Lung 73	BAC, grade 1	cyt, 3	cyt, 1	cyt, 2	cyt, 1	cyt, 2		cyt, 1		cyt, 1				cyt, 1
8	Lung 38	SCC, grade 3	cyt/mem, 3	cyt/mem, 2		cyt/mem, 3	cyt, 1				cyt, 1	cyt, 3	cyt/mem, 3	cyt, 2	cyt/mem, 1
9	Lung 5	SCC, grade 3	cyt/mem, 3	cyt, 1		cyt, 1					cyt/mem, 2	cyt, 1	cyt/mem, 2	cyt, 2	
10	Lung 14	carcioid	cyt, 3			cyt, 1	cyt, 2		cyt, 1	cyt, 2			cyt, 2		
11	Lung 94	carcioid	cyt, 2		cyt, 1		cyt, 1			cyt, 3		cyt, 1			
12	Lung 58	AC, grade 1-2	cyt/mem, 2			cyt, 1			cyt, 1				cyt, 1		
13	Lung 77	BAC, grade 1	cyt, 2	cyt, 1	cyt, 3	cyt, 2	cyt, 3		cyt, 2		cyt,1				
14	Lung 75	BAC, grade 1	cyt, 3		cyt, 2	cyt, 2	cyt, 3		cyt, 2						
15	Lung 91	carcioid	cyt, 3	cyt, 3	cyt, 3					cyt, 3					
16	Lung 52	AC, grade 3	cyt, 1		cyt, 1	cyt, 1	cyt, 1		cyt, 1						

Table 1.
IHC staining of human lung cancer tissue samples showed correlation of high phospho-tyrosine staining and high phospho-Receptor Tyrosine Kinase staining.

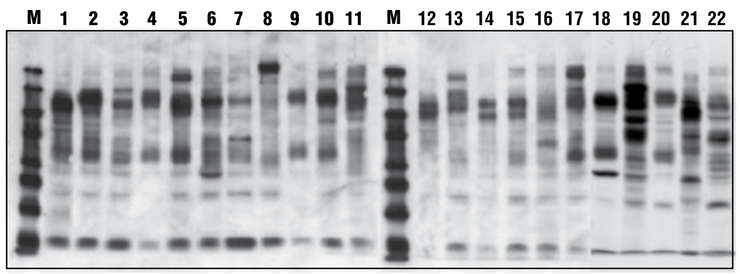

Figure 2.
Western blot analysis of NSCLC cell lines showed heterogeneous phospho-tyrosine signaling in different cells.

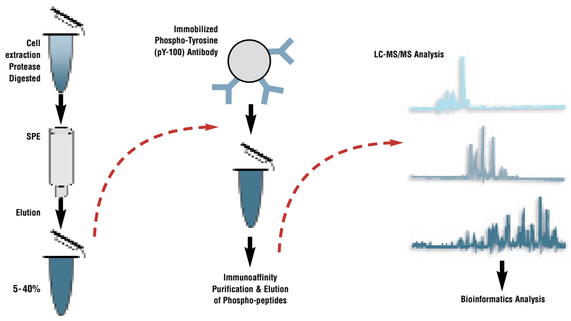

Figure 3. PhosphoScan™ method. Patent Pending (U.S. Patent Publication 20030044848)
Rush, J. et al. (2005) *Nature Biotech.* 23, 94–101.

Cell lines	# analyzed	pY sites	pY sites in kinases	Total Kinases
NSCLC	19	1,159	172	88
SCLC	10	359	78	47

Tumor samples	# analyzed	Normal Control	pY sites nr	pY sites in kinases	Total Kinases
NSCLC	9	7	1,173	78	58
SCLC	2	2	145	25	20

Table 2.
Summary of phospho-tyrosine PhosphoScan™ results from lung cancer cell lines and tumor tissue.

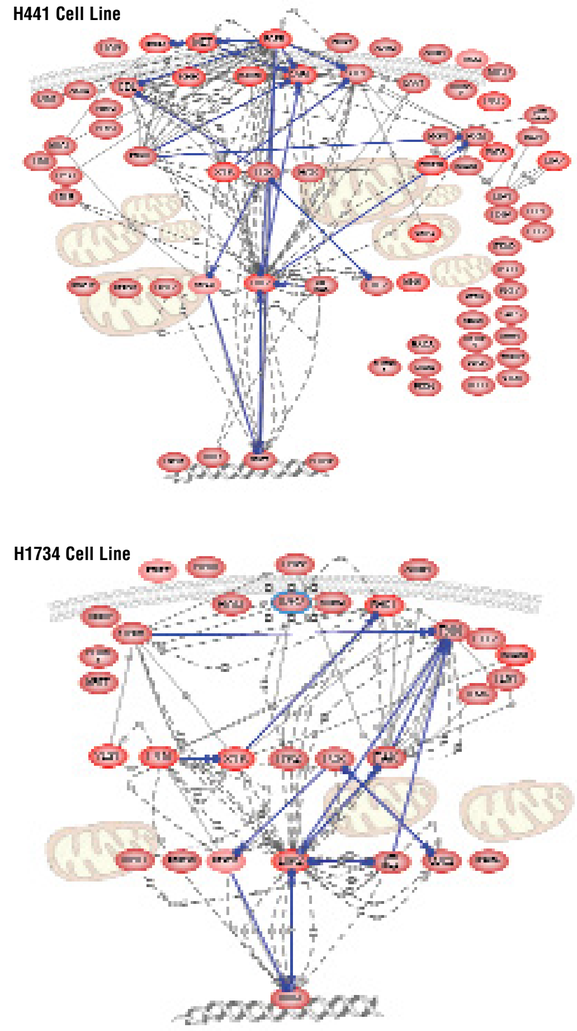

Figure 4. Pathway Map of tyrosine phospho-protein identified by PhosphoScan™ from two different cell lines.

Conclusion

As part of our ongoing efforts to profile tyrosine phosphorylation in lung cancer, we have used our **PhosphoScan™** technology to analyze 19 NSCLC and 10 SCLC cell lines, as well as tumor and normal matched control tissue from patient frozen samples. We have identified in these studies over 2,000 tyrosine phosphorylation sites present in those samples, the majority of which are novel. Among them, we found sites in the activation loops of many protein kinases suggesting that these enzymes and their downstream signaling pathways are activated in lung cancer. Western blotting was used to confirm many activated and phosphorylated proteins in these cell lines. These results have identified novel and complex networks of tyrosine phosphorylated proteins in NSCLC and SCLC. Pathways associated with cell adhesion, cell motility, and receptor signaling are the most prominent features observed in some of cancer cell lines and tumor samples. These results provide the deepest look yet into tyrosine kinase signaling in lung cancer. Using this approach we have identified novel disease drivers as well as potential biomarkers of aberrant kinase activation.

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