

Profiling Tyrosine Phosphorylation in Non Small Cell Lung Cancer

Klarisa Rikova, Ailan Guo, Qingfu Zeng, John Rush, Roberto Polakiewicz, Michael Comb
Cell Signaling Technology, Inc., Beverly, MA.

Introduction

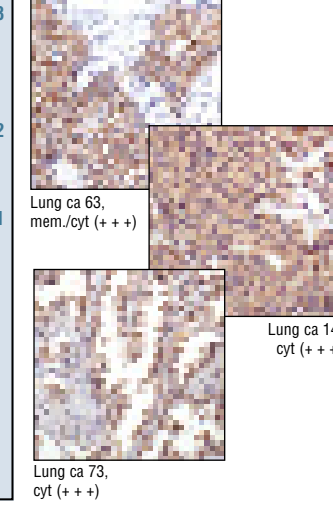
Although NSCLC represents a major cause of cancer related deaths, the genetic alterations that cause and drive this disease are still poorly understood. Recently, mutations in the kinase domains of EGFR and HER2 receptor tyrosine kinases have been identified and in the case of EGFR, the presence of mutant receptor in NSCLC correlates with response to the EGFR inhibitor Irressa. In this study we have investigated the role of activated tyrosine kinases and aberrant tyrosine signaling networks in NSCLC cell lines. We have developed a new method to broadly characterize activated tyrosine kinases and their downstream substrates and apply the method here to NSCLC cell lines. The method involves immunoaffinity purification of tyrosine phosphorylated peptides combined with Mass Spectrometry to identify tyrosine phosphorylated peptides.

Profiling of pY in Non Small Cell Lung Cancer Patients by IHC.

The relationship between p-Tyrosine and p-RTKs IHC staining in Non Small Cell Lung Cancer (Localization and score of IHC staining).

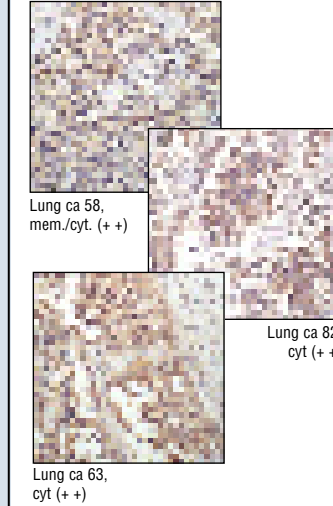
NO	CST Name	ID	9411, p-Tyrosine	p-EGFR				p-HER2			Other p-RTKs				
				2235, p-EGFR (Y992)	2237, P-EGFR (Y1045)	Y1173, P-EGFR (Y1173)	2236, P-EGFR (Y1068)	4404, p-EGFR (Y1148)	2245, P-HER2 (Y1112)	2244, P-HER2 (Y1248)	2249, P-HER2 (Y1221/2)	3151, P-M-CSFR (Y723)	3126, P-Met (Y1234/5)	3161, p-PDGFR (Y751)	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, 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, 1	cyt, 2	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, 2	cyt, 3	cyt, 1	cyt, 1	cyt/mem, 3	cyt, 1	cyt, 1
5	Lung 63	AC, grade 3	cyt/mem, 3	cyt/mem, 3	cyt/mem, 3	cyt/mem, 3	cyt/mem, 3	cyt/mem, 3	cyt/mem, 1	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	cyt, 1	mem, 1	cyt, 1	cyt, 1	cyt, 1	cyt/mem, 2	cyt/mem, 1	cyt/mem, 1	cyt, 1	cyt, 1
7	Lung 73	BAC, grade 1	cyt, 3	cyt, 1	cyt, 2	cyt, 1	cyt, 2	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 3	cyt, 3	cyt, 3	cyt, 1
8	Lung 38	SCC, grade 3	cyt/mem, 3	cyt/mem, 2	cyt/mem, 3	cyt, 1	cyt, 1	cyt, 1	cyt, 1	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, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt/mem, 2	cyt, 1	cyt/mem, 2	cyt, 2	cyt/mem, 1
10	Lung 14	carcioid	cyt, 3	cyt, 1	cyt, 1	cyt, 1	cyt, 2	cyt, 2	cyt, 1	cyt, 2	cyt, 1	cyt, 1	cyt, 2	cyt, 2	cyt, 1
11	Lung 94	carcioid	cyt, 2	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 3	cyt, 1	cyt, 1	cyt, 2	cyt, 2	cyt, 1
12	Lung 58	AC, grade 1-2	cyt/mem, 2	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	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, 2	cyt, 2	cyt, 1	cyt, 1	cyt, 3	cyt, 1	cyt/mem, 1
14	Lung 75	BAC, grade 1	cyt, 3	cyt, 3	cyt, 2	cyt, 2	cyt, 3	cyt, 3	cyt, 2	cyt, 2	cyt, 1	cyt, 1	cyt/mem, 2	cyt, 2	cyt/mem, 1
15	Lung 91	carcioid	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3
16	Lung 52	AC, grade 3	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1
17	Lung 80	BAC, grade 1	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3	cyt, 3

High Staining • Score 3



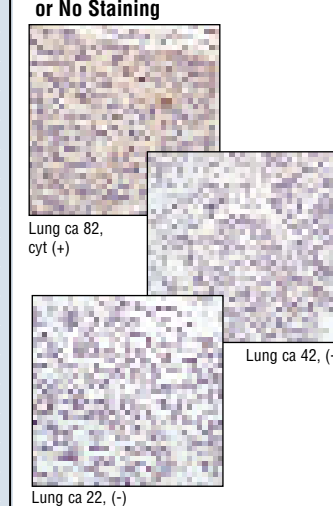
NO	CST Name	ID	9411, p-Tyrosine	p-EGFR				p-HER2			Other p-RTKs				
				2235, p-EGFR (Y992)	2237, P-EGFR (Y1045)	Y1173, P-EGFR (Y1173)	2236, P-EGFR (Y1068)	4404, p-EGFR (Y1148)	2245, P-HER2 (Y1112)	2244, P-HER2 (Y1248)	2249, P-HER2 (Y1221/2)	3151, P-M-CSFR (Y723)	3126, P-Met (Y1234/5)	3161, p-PDGFR (Y751)	3391, p-c-Kit (Y719)
18	Lung 68	AC, grade 1	cyt, 1	cyt/mem, 2	cyt, 1	cyt, 2	cyt, 1					cyt, 1	cyt/mem, 1		cyt, 1
19	Lung 53	AC, grade 3	cyt/mem, 3		cyt, 1		cyt, 1					cyt/mem, 1			
20	Lung 17	SCC, grade 2	cyt, 2			cyt, 1						cyt, 2			
21	Lung 83	BAC grade 1	cyt, 3			cyt, 1						cyt, 2			
22	Lung 4	SCC, grade 2	cyt, 1		cyt, 2		cyt, 1					cyt, 1			
23	Lung 6	AC with BAC, grade 1-2	cyt, 2				cyt, 1					cyt, 1			
24	Lung 8	AC, grade 3-1	cyt, 2				cyt, 3					cyt, 3			
25	Lung 9	AC, grade 3	cyt, 2		cyt, 2							cyt, 1			
26	Lung 19	AC, grade 2				cyt, 1									
27	Lung 40	SCC, grade 1-2	cyt, 1		cyt, 1										
28	Lung 49	SCC, grade 2	cyt, 1		cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1	cyt, 1
29	Lung 60	BAC, grade 1	cyt, 2				cyt, 3								
30	Lung 61	AC, grade 2	cyt, 1		cyt, 1										
31	Lung 78	BAC, grade 2	cyt, 3		cyt, 1										
32	Lung 99	SCC, grade 1-2	cyt, 1							cyt, 1					
33	Lung 70	AC, grade 2	cyt/mem, 1							cyt, 1		cyt, 1			
34	Lung 95	carcioid	cyt, 1							cyt, 1					
35	Lung 82	AC with BAC, grade 2-3	cyt, 2							cyt, 1					
36	Lung 86	BAC, grade 1-2	cyt, 2							cyt, 1					

Mid Staining • Score 2



NO	CST Name	ID	9411, p-Tyrosine	p-EGFR				p-HER2			Other p-RTKs				
				2235, p-EGFR (Y992)	2237, P-EGFR (Y1045)	Y1173, P-EGFR (Y1173)	2236, P-EGFR (Y1068)	4404, p-EGFR (Y1148)	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)
37	Lung 3	SCC, grade 1	cyt, 1									cyt, 2			
38	Lung 36	SCC, grade 1-2	cyt, 1									cyt, 2			
39	Lung 62	AC, grade 2	cyt, 1												
40	Lung 64	AC, grade 3	cyt, 1												
41	Lung 43	SCC, grade 2-3	cyt, 2												
42	Lung 26	SCC, grade 3	cyt, 2												
43	Lung 27	SCC, grade 2	cyt, 1												
44	Lung 28	SCC, grade 2-3	cyt, 1												
45	Lung 30	SCC, grade 2-3	cyt, 1												
46	Lung 31	SCC, grade 2-3	cyt, 1												
47	Lung 15	AC with BAC, grade 1	cyt, 1												
48	Lung 23	SCC, grade 1	cyt, 1												
49	Lung 24	SCC, grade 2	cyt, 1												
50	Lung 25	SCC, grade 2-3	cyt/mem, 2												
51	Lung 67	AC with BAC, grade 1	cyt, 1												
52	Lung 71	AC, grade 2	cyt, 1												
53	Lung 72	AC, grade 3	cyt, 1												
54	Lung 51	SCC, grade 3	cyt, 1												

Low Staining • Score 1 or No Staining

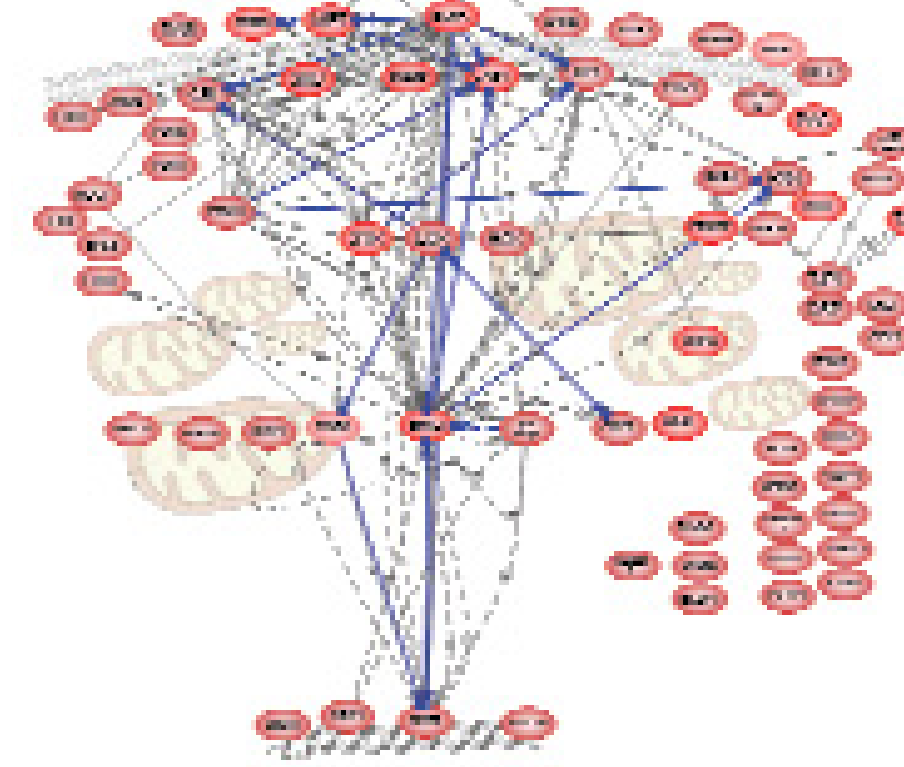


Conclusion

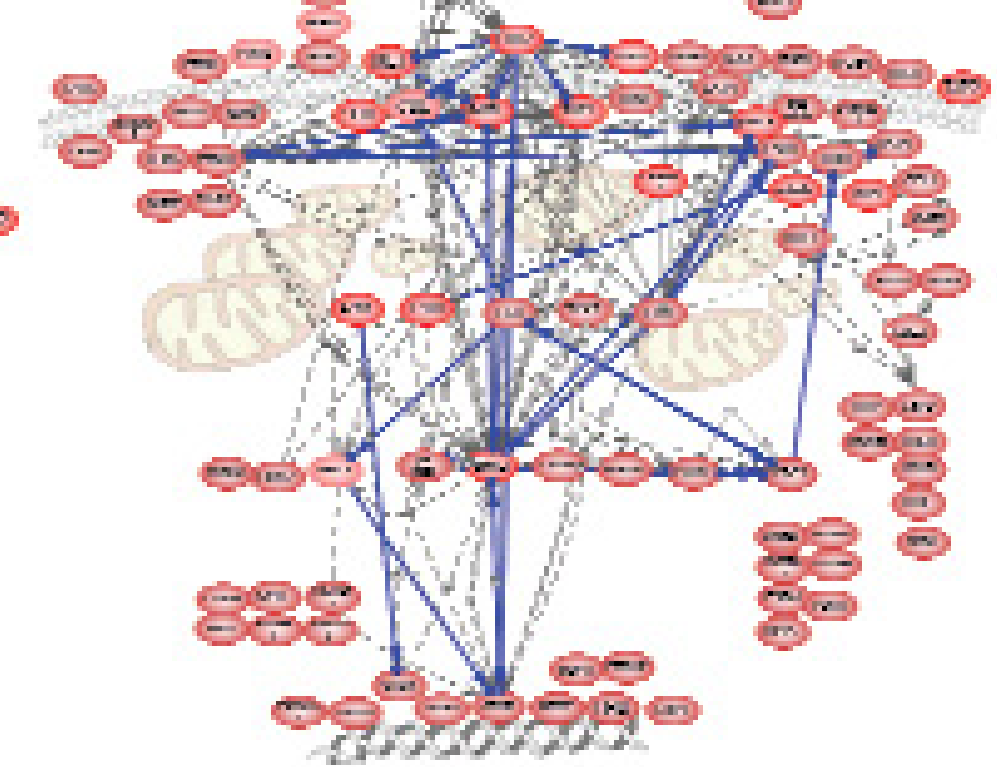
Here we present phosphotyrosine analysis of four different NSCLC cell lines. Many tyrosine, serine and threonine kinases were found to be tyrosine phosphorylated at their activation loops suggesting that these enzymes and their downstream signaling pathways are active. Western blotting was used to confirm many activated and phosphorylated proteins in these cell lines. The results identify complex networks of tyrosine phosphorylated proteins in NSCLC. Pathways associated with cell adhesion, cell motility, and extracellular receptor signaling are the most prominent features observed in the cancer cell lines. These results provide the deepest look yet into tyrosine kinase signaling in NSCLC and identifying the disease drivers.

Pathway maps showing the relationship of phospho-proteins identified P-Tyr immunoaffinity profiling.

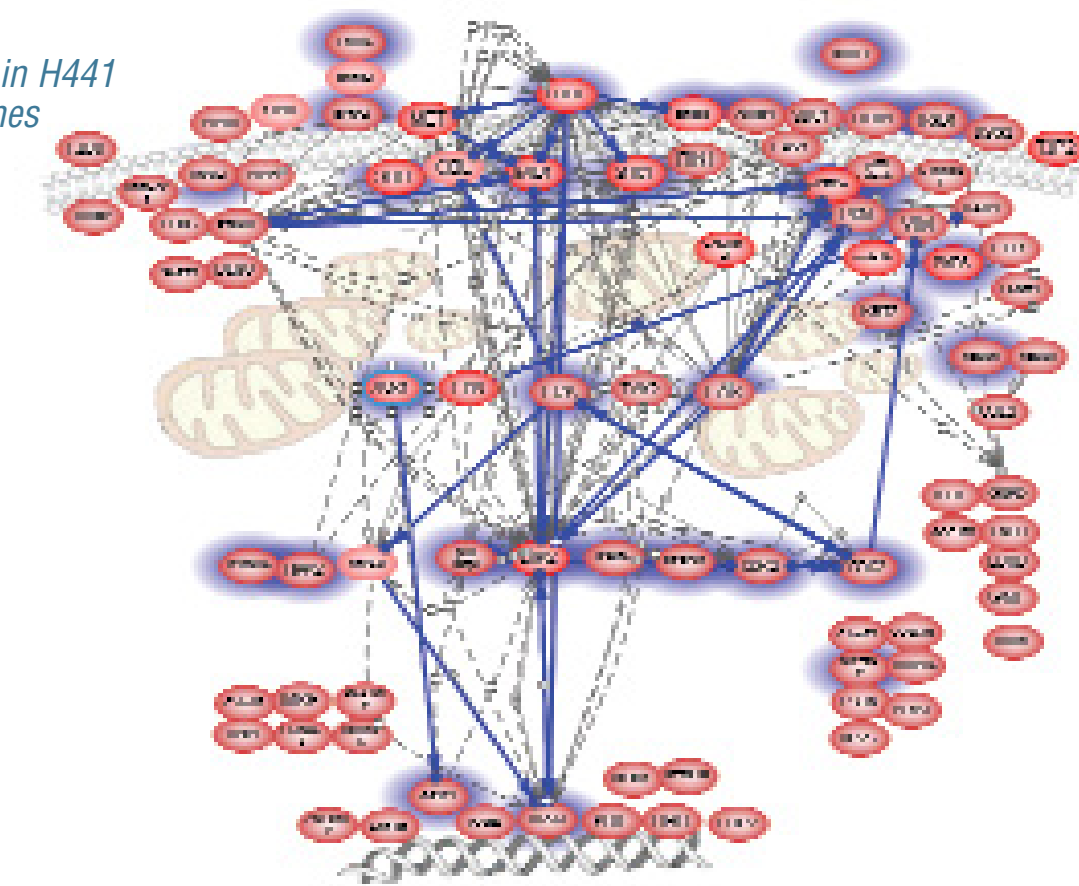
H441 Cell Line



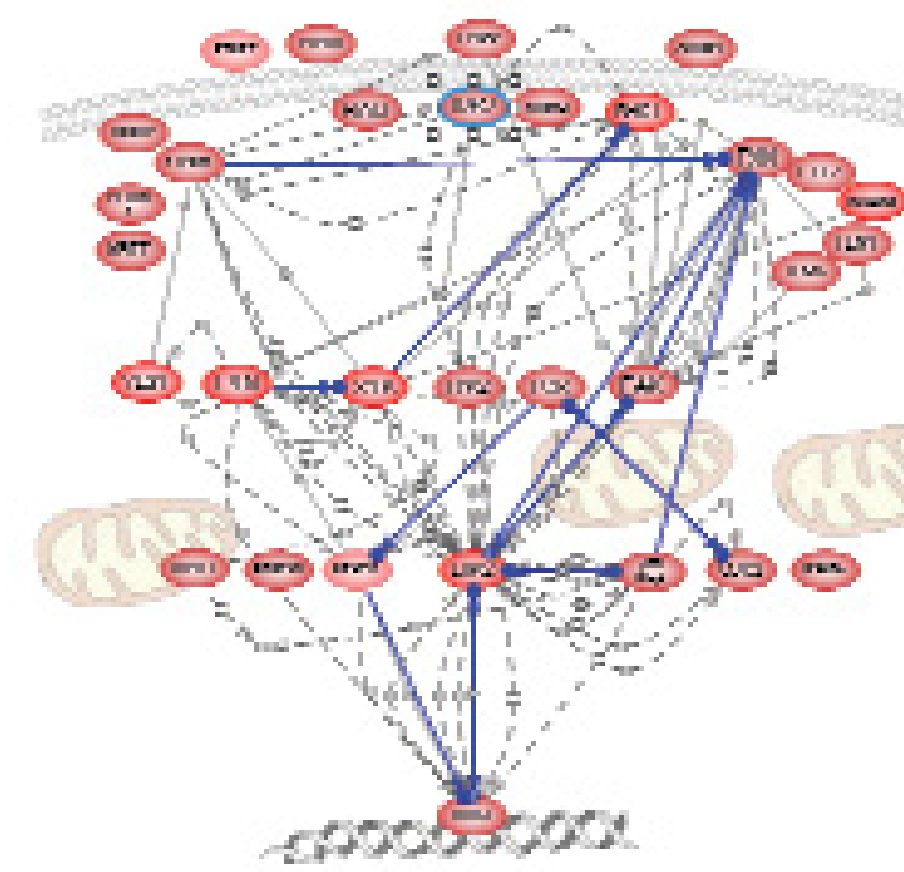
H1373 Cell Line



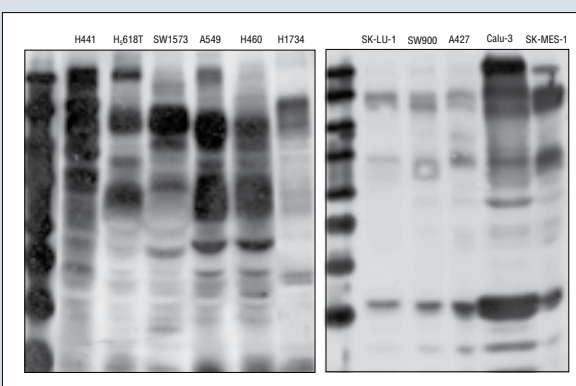
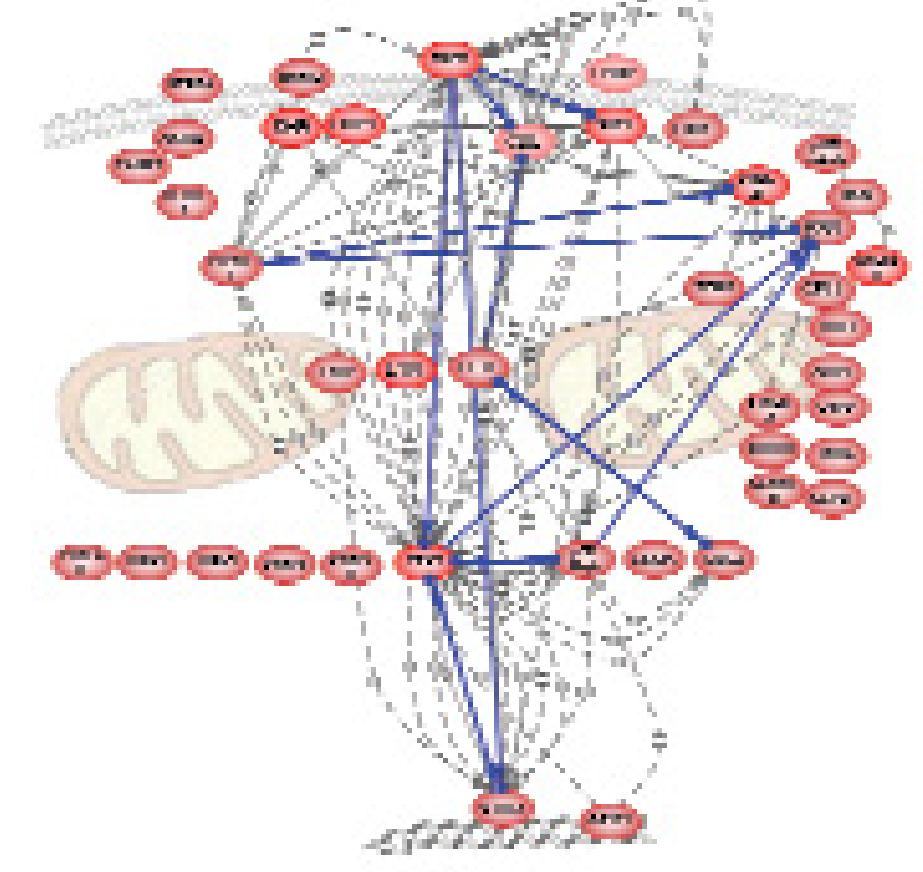
Common proteins in H441 and H1373 Cell Lines



H1734 Cell Line



SK-MES-1 Cell Line



Western blot analysis of Non Small Cell Lung Cancer Cell Lines starved over night with pY antibody.