Analysis of Bcr/Abl signaling using large panels of phospho-sensitive antibodies

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Abstract

The Bcr/Abl fusion tyrosine kinase is constitutively active and is associated with CML and ALL. In this study, we analyzed cell signaling in a number of untreated and Gleevec-treated Bcr/Abl expressing cells lines (b3a2, b2a2, and e1a2 isoforms) by flow cytometry using a broad panel of phospho-sensitive antibodies to determine the degree of signaling heterogeneity in model cells lines. Using this paradigm, we identified a number of phosphoproteins involved in the Ras/MAPK, PI3K/Akt, and Jak/Stat pathways, including c-Cbl, CrkL, Erk, FAK, Shc, SHP-2, and Stat5, that were constitutively activated in untreated cells and markedly decreased by Gleevec treatment. Interestingly, there were differences in the signaling profiles among the cell lines. These differences may represent differences among the Bcr/Abl signaling pathways and may have significance for disease treatment. These results demonstrate the use of phospho-specific antibodies as tools to analyze complex signaling in leukemic cells. This type of analysis may lead to the identification of signaling proteins that are robust biomarkers and/or potential therapeutic targets.

Methods

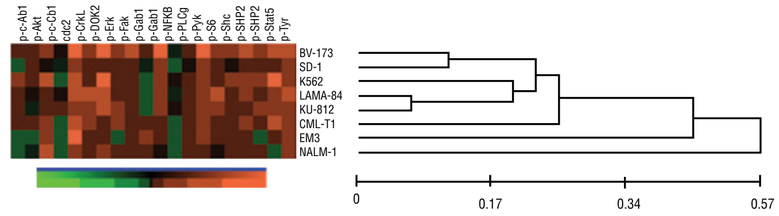
Eight cell lines expressing different Bcr/Abl fusion isoforms (e1a2, b2a2, b3a2) were treated with Gleevec (10 uM) for 60 min, then fixed with 2% formaldehyde and permeabilized with 90% methanol. Treated and untreated cells were screened by flow cytometry using phospho-specific antibodies to identify signaling proteins that were affected by the Gleevec treatment.

Cell Line	Bcr/Abl Isoform
SD-1	e1a2
BV-173	b2a2
CML-T1	b2a2
EM3	b3a2
K562	b3a2
KU-812	b3a2
LAMA-84	b3a2
NALM-1	b3a2

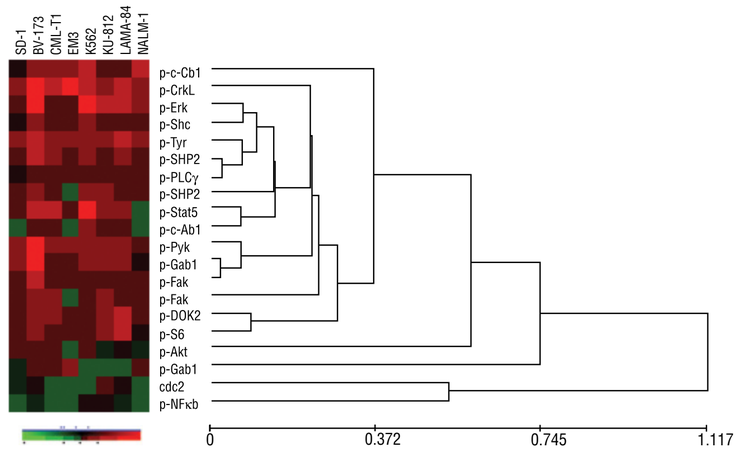
Antibodies Used

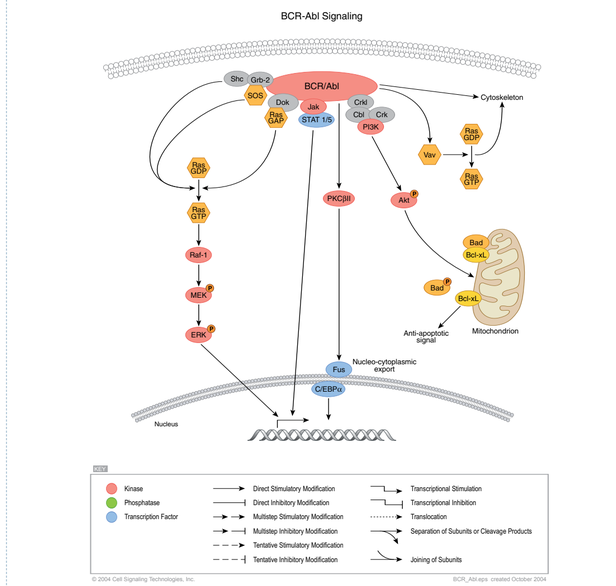
Phospho-c-Abl (Tyr245)	Phospho-FAK (Tyr576/577)	Phospho-Shc (Tyr317)
Phospho-Akt (Ser473)	Phospho-FAK (Tyr925)	Phospho-SHP-2 (Tyr542)
Phospho-c-Cbl (Tyr774)	Phospho-Gab1 (Tyr627)	Phospho-SHP-2 (Tyr580)
cdc2	Phospho-Gab1 (Tyr307)	Phospho-Stat5 (Tyr694)
Phospho-CrkL (Tyr207)	Phospho-NF-κB p65 (Ser536)	Phospho-Tyrosine (P-Y100)
Phospho-p56 DOK2	Phospho-PLCg1 (Tyr783)	
Phospho-p44/42 MAPK (Thr202/Tyr204)	Phospho-Pyk2 (Tyr402)	
	Phospho-S6 Ribosomal Protein (Ser235/236)	

Cluster analysis of eight Bcr/Abl cell lines. Cell lines were clustered by average distance according to fold inhibition by Gleevec of protein phosphorylation or expression.



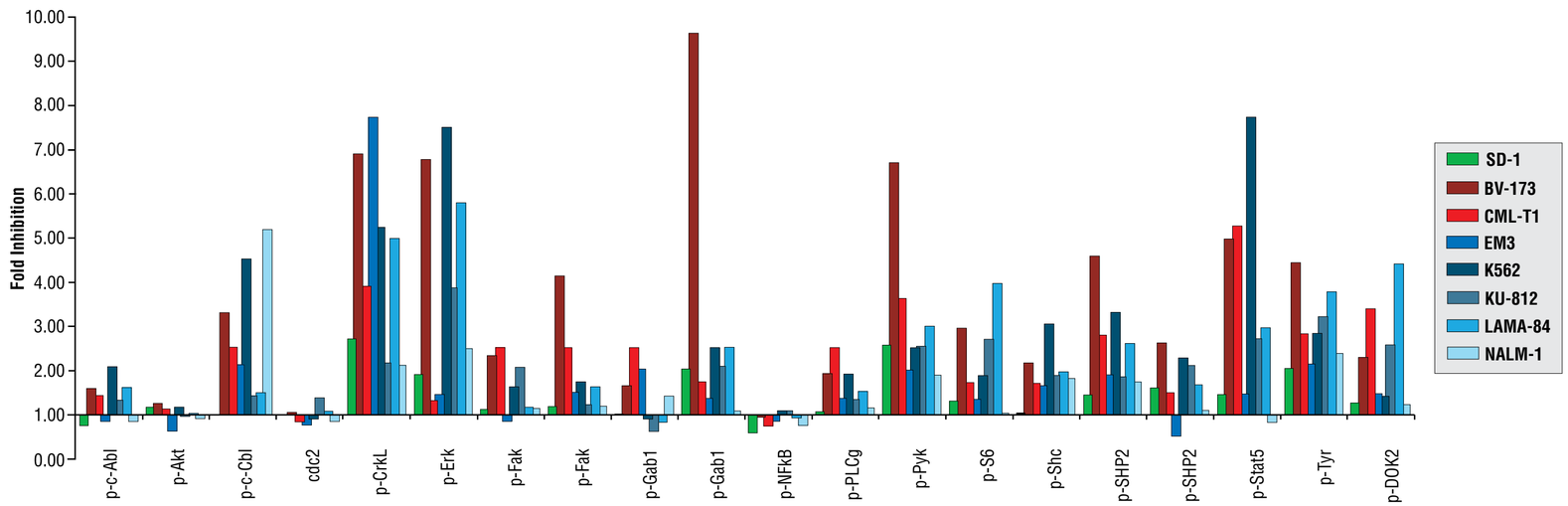
Cluster analysis of protein phosphorylation/expression in eight Bcr/Abl cell lines. Proteins were clustered by average distance according to fold inhibition by Gleevec of protein phosphorylation.





Cell-Specific Gleevec Inhibition

Analysis of Gleevec-specific changes in phosphorylation or expression of twenty signaling proteins in eight Bcr/Abl cell lines.



Conclusions

- The degree of inhibition of phosphorylation of signaling proteins varies widely in the Bcr/Abl cell lines studied.
- There were no clear Bcr/Abl isoform-specific signaling profiles. The targets studied were generally more inhibited by Gleevec in CML cell lines (b2a2 and b3a2) than in the ALL cell line (e1a2). However, only one ALL cell line was examined in this study.
- Though most targets were dephosphorylated by Gleevec, NFkB was more phosphorylated following Gleevec treatment in most cell lines.

These results indicate that different Bcr/Abl cell lines have very different signaling patterns in response to Gleevec, and highlights the importance of using multiple cell lines to examine leukemic signaling in cell lines.

This method of using phospho-specific antibodies to analyze complex signaling in cancer cells may lead to the identification of signaling proteins that are robust biomarkers and/or potential therapeutic targets, and also provides researchers and clinicians with a tool to analyze therapeutic efficacy in cancer cells.