Detection of an Extremely Rare BCR-ABL Fusion in H929 Multiple Myeloma Cells Using a Label-Free IP-LC/MS/MS Proteomics Approach

Susanne B. Breitkopf, Min Yuan, German Pihan, and John M. Asara

1. Division of Signal Transduction, Beth Israel Deaconess Medical Center, Boston, MA; 2. Department of Medicine, Harvard Medical School, Boston, MA; 3. Department of Hematology, Beth Israel Deaconess Medical Center, Boston, MA

Introduction
Protein-protein interaction (PPI) networks in addition to the phosphoproteome are important for understanding the function and regulation of pathways leading to cell growth and proliferation of cancers cells. The advantage of analyzing purified protein complexes is to identify differentially interacting proteins and post-translational modifications that might otherwise go undetected in large-scale analyses. This can be achieved by performing immunoprecipitation (IPs) with antibodies against the bait protein and analyzing the prey interacting proteins by tandem mass spectrometry (LC-MS/MS). Here, we show an example of using a three-pronged IP-MS technology (p85 [PI3K], GRB2, and pY) in H929 multiple myeloma (MM) cells and how we identified an extremely rare BCR-ABL fusion in MM by the identification of converging protein-protein interaction networks and activated tyrosine kinases. We also show that imatinib selectively inhibits signaling and cell proliferation in H929 cells.

How to choose the treatment for a cancer patient with "smart drugs"

"MS Assays"

The canonical BCR-ABL fusion pathway in CML highlighting identified proteins from the integrated 3-pronged IP-LC/MS/MS network

In H929 cells, 12 proteins overlapped in at least two of the three IP-MS experiments including proteins like BCR, ABL1, SHP2, CBL, and GRB2. We have shown that pY and pY immunoblot of H929 WCL after 1 hr incubation with various TKI drugs including imatinib and the proteasome inhibitor bortezomib. Imatinib treated H929 cell lines show selective inhibition of imatinib on pY, pSFK, and pS6K levels.

Proteomics

Conclusions

Three IP [p85, GRB2, and pY] combined with tandem mass spectrometry (LC-MS/MS) is used to aid in treatment decisions using "smart" drug kinase inhibitor therapy.

Targeted LC/MS/MS

Targeted Proteomics based on immunoprecipitation (IP) and shotgun tandem mass spectrometry (LC-MS/MS) is used to aid in treatment decisions using "smart" drug kinase inhibitor therapy.

Methods

- Cells were fixed and 10 mg of protein was used in immunoprecipitation (IP) and the GRB2 adapter using anti-phospho antibodies, followed by trypsin digestion.
- ECL was used to detect specific bands.

References


Pathscan RTK Signaling assay kit tests the phospho-Tyr status of 39 different kinases. H929 MM cell cultures were stimulated for 0, 2, 5, and 10 min with insulin, insulin growth factor (IGF), epidermal growth factor (EGF), and 10% fetal bovine serum (FBS) or serum starved. Few kinases showed high phosphorylation signals including S6K and AKT. Data support the proteomics results indicating that ABL is driving H929 cell proliferation.

Pathscan RTK Signaling assay kit tests the phospho-Tyr status of 39 different kinases. H929 MM cell cultures were stimulated for 0, 2, 5, and 10 min with insulin, insulin growth factor (IGF), epidermal growth factor (EGF), and 10% fetal bovine serum (FBS) or serum starved. Few kinases showed high phosphorylation signals including S6K and AKT. Data support the proteomics results indicating that ABL is driving H929 cell proliferation.

Data suggests testing multiple myeloma patients for common genetic mutations such as BCR-ABL as part of the routine diagnosis.

Acknowledgments

We thank Constantine Mitsiades and Kenneth Anderson (DFCI) for helpful discussions and for donating H929 cells. We also thank Shang Li for donating MM-COOH, MM-2665, MM-1208, and MM-1208 cell lines. Alex Almassi (TCCI) for donating H929 cells and the lab members of Lewis Center (BIDMC) for helpful discussions. We acknowledge NIH grants P50CA115810, P50CA129629, and U54CA119356 from the National Cancer Institute for support.

Histopathology report of H929 MM cells compared to neoplastic plasma cells and CML cells

H929 MM cells were processed for histology and immunohistochemistry. H929 MM cells were stained with BCR-ABL, B2M, and Cytokeratin. The obtained DNA and translated protein sequence aligned with the common and imatinib sensitive BCR-ABL fusion.

DNA sequencing analysis of BCR-ABL transcritp in H929 cells

DNA sequencing was used to identify the BCR-ABL fusion in H929 multiple myeloma cells. Reverse-transcription (RT)-PCR with primer pairs specific for different forms of BCR-ABL fusion was performed on H929 cells to identify the specific sequence of the novel fusion. 

DNA sequencing was used to identify the BCR-ABL fusion in H929 multiple myeloma cells. Reverse-transcription (RT)-PCR with primer pairs specific for different forms of BCR-ABL fusion was performed on H929 cells to identify the specific sequence of the novel fusion. 

A) Two prominent DNA bands were extracted from the agarose gel and sequenced by the Sanger method. B) The obtained DNA and translated protein sequence aligned with the common and imatinib sensitive e14a2 form of BCR-ABL fusion.

Conclusion

- Discovery led to selective phosphotyrosine inhibition by ABL1 inhibitor (Gleevec) leading to cell death.
- Different lots of H929 cells from different vendors (DSMZ, Germany and ATCC, USA) and academic laboratories showed that BCR-ABL was present in all ATCC cells to varying levels.
- The label-free SHP2 IP-MS experiment from H929 cells treated for 1hr with 500nM imatinib and untreated (DMISO) to test the imatinib effect on the SHP2 protein-protein interaction (PP) complex in BCR-ABL fused cells. SHP2 binding strength was determined by the number of spectral counts and average of the Top 3 total ion current (TIC) values for each interacting protein. Results showed that SHP2 and p110 dissociate from SHP2 complex and BCR to agrobate pERK and pAKT, revealing a model.

In H929 cells, 12 proteins overlapped in at least two of the three IP-MS experiments including proteins like BCR, ABL1, SHP2, CBL, and GRB2. We have shown that pY and pY immunoblot of H929 WCL after 1 hr incubation with various TKI drugs including imatinib and the proteasome inhibitor bortezomib. Imatinib treated H929 cell lines show selective inhibition of imatinib on pY, pS6K, and pS6K levels.