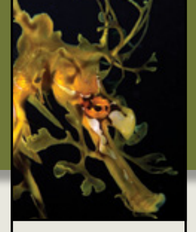

Detection of Genetic Abnormalities in Cancer Cells using Bio-Plex® Immuno-Assays

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Introduction

Receptor tyrosine kinases initiate a cooperative signaling network responsible for regulating multiple biological processes. Aberrant activation of such networks has been shown to play significant roles in a variety of cancers. Genetic abnormalities deregulate these tightly controlled networks and confer altered responses to drugs. For example, mutations in the EGFR gene leads to increased sensitivity or resistance to EGFR-targeted drugs and translocations within the Bcr-Abl gene abolish responsiveness to Gleevec treatment in chronic myeloid leukemia (CML). Assays that can detect such abnormalities in patient samples would facilitate selection of the optimal treatment regimen and enable monitoring of the cancer during therapy. In this study we demonstrate the specific detection of an EGFR point mutation and deletion and Bcr-Abl translocations in cancer cells using Bio-Plex® bead-based immunoassays.

Methods

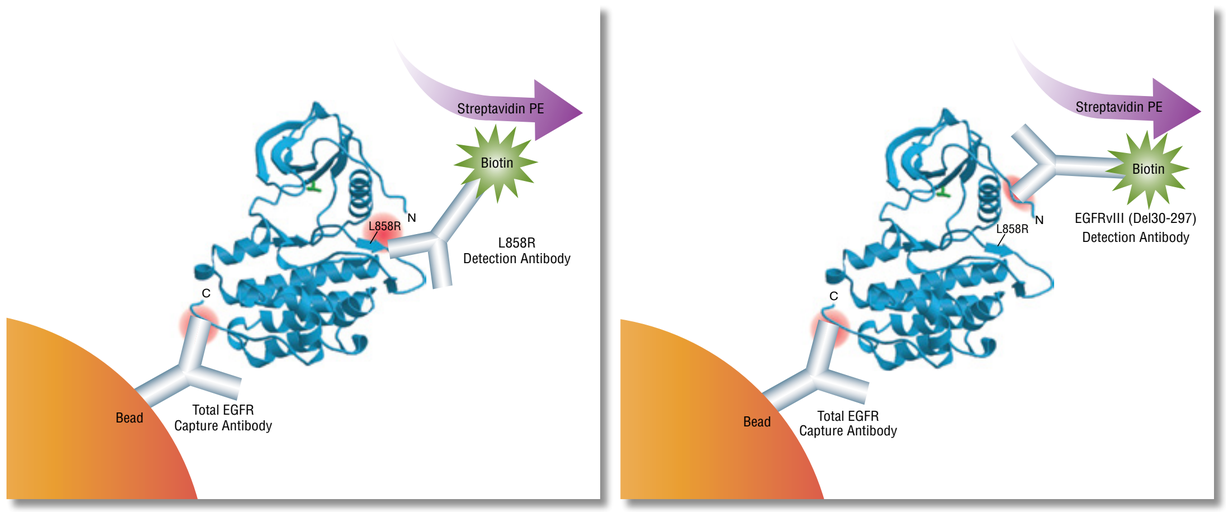
Bio-Plex® Assays: Three Bio-Plex® immunoassays were developed that detect either a specific point mutation or deletion in EGFR, or fusion proteins resulting from the different translocations of the Bcr-Abl gene. The EGFR mutation or deletion assays were developed using antibodies reactive with EGFR regardless of the mutation status (capture) and antibodies specific for the aberrant form of the receptor (detection). Bcr-Abl was captured with an antibody to Bcr and detected with anti c-Abl antibody. All antibodies were from Cell Signaling Technology. Reactions were performed in 96-well, filter-bottomed plates. 2500 Bio-Plex® beads coated with the appropriate capture antibody were added to the plate and incubated overnight with the various lysates. Following washing, the detection antibodies were added and the plates incubated for 30 minutes. The beads were then washed and incubated for 5 minutes in Streptavidin-conjugated R-phycoerythrin. The beads were washed again and analyzed using the Bio-Plex® machine. Western blot analyses of cell lysates were performed following protein separation by SDS-PAGE and transfer to nitrocellulose membranes.

Cell Lines: The human CML cell lines K562, SUP-15 and CML-T1 and the non-small cell lung cancer cell lines NCI-H358 and NCI-H1975 were purchased from ATCC. The 3T3 cell line transfected with EGFRvIII (Del30-297) was genetically engineered at the Broad Institute of MIT and Harvard.

Results

EGFR mutations: Antibody pairs able to distinguish between different EGFR mutations (Fig. 1) were identified and characterized.

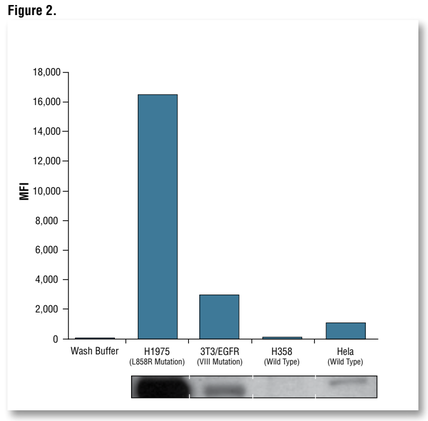
Figure 1.



Schematic representation of mutation specific EGFR antibody pair binding for L858R point mutation (left) and EGFRvIII deletion mutation (right).

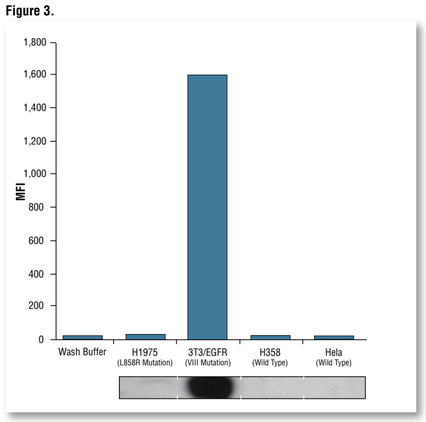
EGFR mutations: Fig. 2 depicts the L858R mutant specific antibody pair tested using cell lines expressing the appropriate point mutation (H1975), deletion mutation (3T3/EGFRvIII, Del30-297), and no mutation (H358 and Hela). The results show preferential recognition of the L858R point mutation in EGFR. When the antibody pair specific for the aa30-297 deletion was tested using the same cell lysates as above, only the lysate prepared from cells expressing the deletion mutant EGFR were detected (Fig. 3). The results corroborated Western blot analysis.

Figure 2.



Detection of L858R mutant EGFR protein in lysates from H1975, 3T3/EGFRvIII, H358 and Hela cells by Bio-Plex® and western blot.

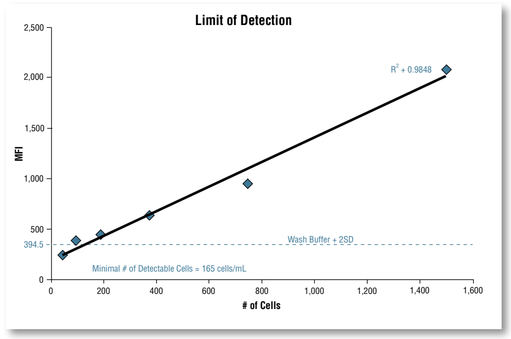
Figure 3.



Detection of EGFRvIII mutant protein in lysates from H1975, 3T3/EGFRvIII, H358 and Hela cells by Bio-Plex® and western blot.

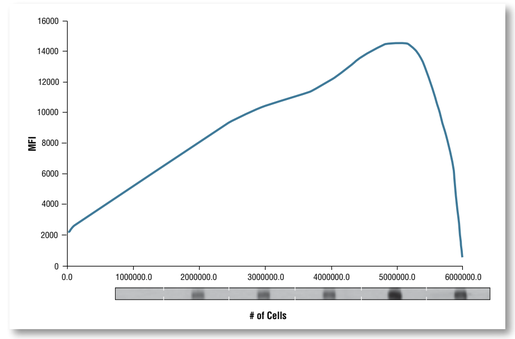
Bcr-Abl Chromosomal Translocations: Limit of detection (LOD) experiments using K562 lysates showed that Bcr-Abl can be detected reliably at levels as low as 165 cells/mL.(Fig. 4A). This is significantly superior to western blot LOD (~ 3 x 106 cells/mL) as seen of Fig.4B. Since CML chromosomal abnormalities result in different Bcr-Abl fusion proteins depending on the length of Bcr being translocated (Fig. 5), we examined cell lines expressing either b2a2 (CML-T1), b3a2 (K562) or e1a2 (SUP-B15) Bcr-Abl isoforms. As shown in fig.6, the antibody pair used in the Bio-Plex immunoassay is able to detect all isoforms. Similar results were obtained in Western blot analysis.

Figure 4A.



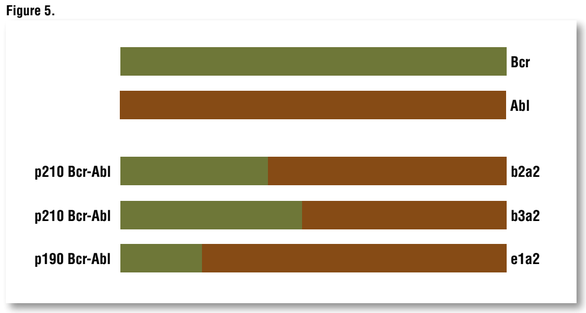
Limit of detection of the number of K562 cells using Bcr-Abl antibody pair.

Figure 4B.



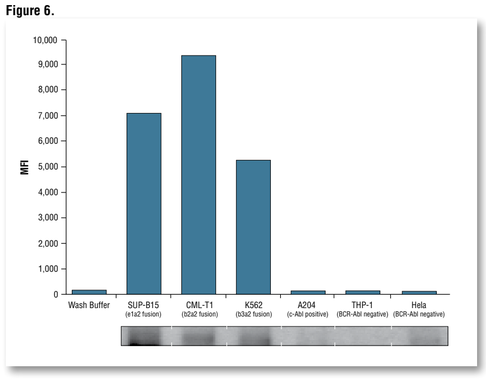
Comparison of Bio-Plex® and Western blot assay sensitivity using varying numbers of k562 cells.

Figure 5.



Schematic representation of different Bcr-Abl fusion proteins.

Figure 6.



Bio-Plex® and Western Blot analysis of CML cell lines representing different Bcr-Abl isoforms.

Conclusions

- Antibody pairs reactive with EGFR point mutation or deletion demonstrated suitable specificity for use in disease management and monitoring treatment.
- The Bio-Plex® immunoassay for the detection of Bcr-Abl translocations was more sensitive than western blotting.
- The Bcr-Abl specific antibody pair recognized all Bcr-Abl isoforms tested.
- We have successfully developed assays capable of detecting genetic abnormalities in cancer cells using specific antibodies and the Bio-Plex® platform. The ability to multiplex genetic analysis will enable the precise and rapid evaluation of the mutational status of research and clinical samples.

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CST and Bio-Rad have an alliance in which CST's expertise is applied to screen and validate capture and detection antibody pairs to expedite Bio-Plex® assay development.

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