

Detection of Genetic Abnormalities in Cancer Cells Using Bio-Plex Immunoassays

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Introduction

Receptor tyrosine kinases initiate a tightly controlled cell-signaling network that regulates multiple biological processes. Aberrant activation of such networks plays significant roles in both development and treatment of a variety of cancers (Amann et al. 2005). Genetic abnormalities can further deregulate these networks and confer altered responses to treatment drugs. For example, mutations in the epidermal growth factor receptor (EGFR) gene can lead to either increased sensitivity or resistance to EGFR-targeted drugs. Likewise, mutations within the Bcr-Abl gene abolish responsiveness to Gleevec (imatinib mesylate, Novartis) treatment in chronic myelogenous leukemia (CML). Assays that can detect such mutations in patient samples would facilitate selection of the optimal treatment regimen and enable monitoring of the cancer during therapy.

We developed two sets of capture and detection antibodies specific to the L858R point mutation in EGFR and to Bcr-Abl translocations. In this study, we demonstrate specificity and, for Bcr-Abl, limits of detection of these aberrations in cancer cells. We use Bio-Plex bead-based immunoassays and western blotting for comparison.

Methods

Bio-Plex Assays

Bio-Plex immunoassays detecting the L858R point mutation in EGFR and fusion proteins resulting from translocations of the Bcr-Abl genes were developed. The EGFR mutation assay was developed using a capture antibody reactive with EGFR regardless of its mutation status, and a detection antibody specific for the aberrant form of the receptor (Figure 1). Bcr-Abl was captured with an antibody to Bcr and detected with anti-c-Abl antibody. Cell Signaling Technology, Inc. provided all antibodies.

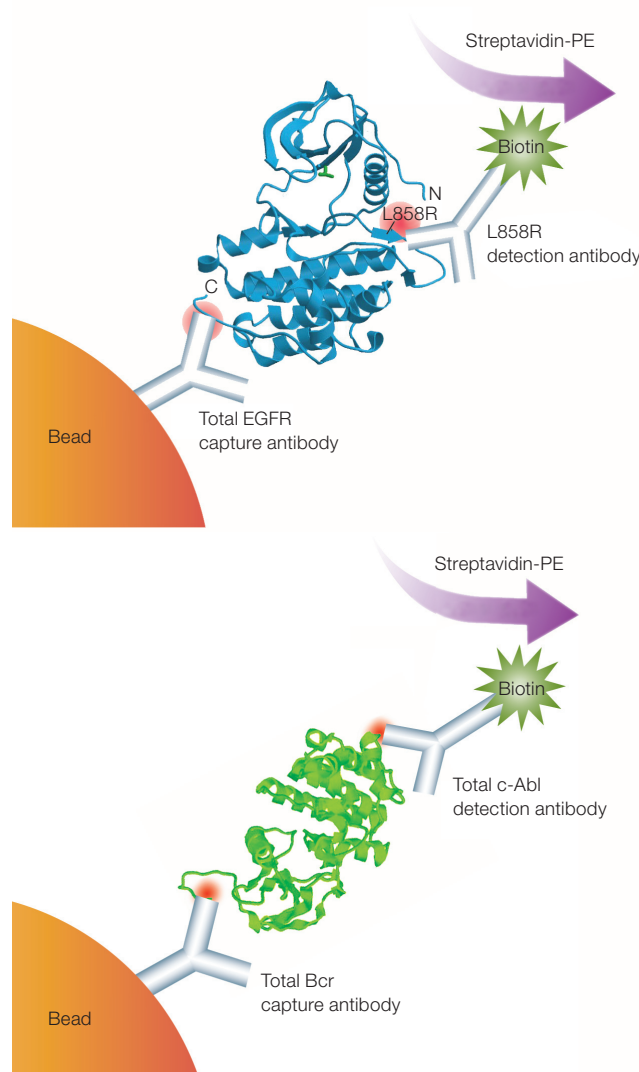


Fig. 1. Method of detection of mutated EGFR (top) and translocated Bcr-Abl (bottom) using Bio-Plex bead-based immunoassays. In each case, detection requires two specific antibodies: one to capture total protein on the bead, the other to detect the specific abnormality. Antibody pairs were developed to detect EGFR mutation at L858R and Bcr-Abl fusion proteins of varying lengths. Figure is not drawn to scale.

Reactions were performed in 96-well, filter-bottomed plates according to the Bio-Plex phosphoprotein detection instruction manual. Briefly, 2,500 beads coated with the appropriate capture antibody were added to each well in the plate and incubated overnight with lysates from one of eight cell lines obtained from American Type Culture Collection (ATCC) (Table 1). Cells were homogenized by sonication in cell lysis buffer. Following washing, detection antibodies were added and the plates incubated for 30 min. The beads were then washed and incubated for 5 min in streptavidin R-conjugated phycoerythrin. The beads were washed again and analyzed using the Bio-Plex system array reader. Western blot analyses of identically treated cell lysates were performed following protein separation by SDS-PAGE and transfer to nitrocellulose membranes.

Chromosomal abnormalities can create a variety of Bcr-Abl translocations. Depending on translocation length, this results in Bcr-Abl fusion proteins of different composition (Figure 2), which can present a challenge to detection by antibodies. To test our antibody performance, we examined three cell lines expressing differing fusion proteins: e1a2, b2a2, and b3a2, as well as c-Abl alone and Bcr-Abl negative as controls (see Table 1 for names of cell lines).

Results and Discussion

EGFR Mutation

The L858R mutant-specific antibody pair preferentially recognized the L858R point mutation in EGFR (Figure 3). The results corroborated western blotting analysis using the L858R mutant-specific antibody.

Bcr-Abl Chromosomal Translocations

Limit of detection, as determined by the minimum detectable number of cells/ml, was calculated using K-562 lysates. The Bio-Plex assays reliably detected Bcr-Abl at levels as low as 165 cells/ml (Figure 4A), about 4 orders of magnitude more sensitive than our results for western blotting ($\sim 2 \times 10^6$ cells/ml, Figure 4B). The Bio-Plex assay detected Bcr-Abl at densities from 165 cells/ml to 5×10^6 cells/ml.

The antibody pair used in the Bio-Plex immunoassay detected all three Bcr-Abl isoforms tested (Figure 5). Western blot analysis using a Bcr-specific antibody yielded similar results.

Table 1. Cell lines used to test detection of EGFR point mutation and Bcr-Abl translocation.

Trait	Cell Line	Cancer Type
EGFR		
Wild type	NCI-H358	NSCLC*
L858R point mutation	NCI-H1975	NSCLC
Bcr-Abl		
No translocation	THP-1	AML*
b2a2**	T1	CML*
b3a2**	K-562	CML
e1a2**	SUP-B15	CML
c-Abl positive	A-204	Rhabdomyosarcoma
EGFR-wild; Bcr-Abl-normal	HeLa	Cervical cancer

* NSCLC, non-small cell lung cancer; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia.

** See Figure 2.

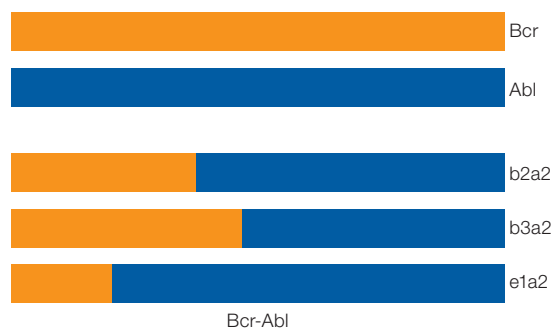


Fig. 2. Bcr-Abl fusion protein isoforms tested in this study.

Translocation can result in fusion proteins containing differing Bcr and Abl lengths. Three such isoforms, b2a2, b3a2, and e1a2, were tested using a single antibody pair.

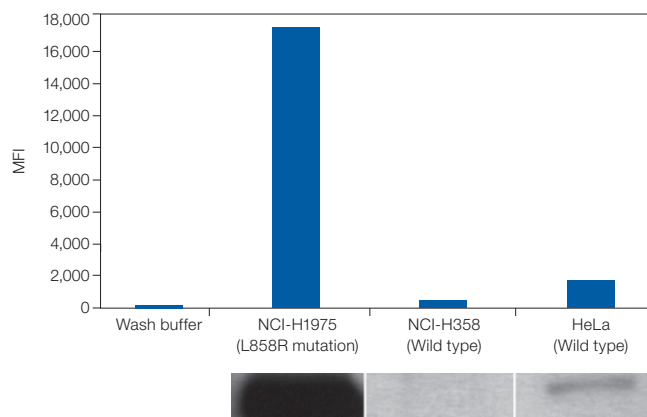


Fig. 3. Detection of the L858R point mutation in EGFR protein in lysates from three cell lines. Line NCI-H1975 harbors the mutation; NCI-H358 and HeLa lines are wild type. Bio-Plex assays (top) and western blotting (bottom) yielded similar results.

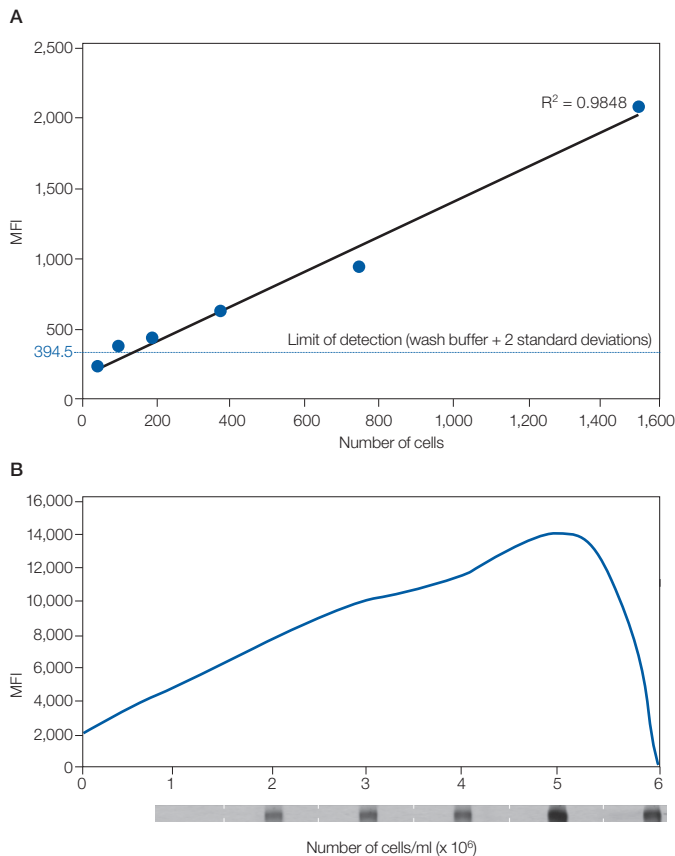


Fig. 4. Limits of detection for Bcr-Abl translocations in K-562 cells. **A**, Bio-Plex assays detected translocations at concentrations as low as 165 cells/ml; **B**, upper limit of detection for Bio-Plex assays (upper scan) was 5×10^6 cells/ml; western blotting (lower image) lower limit of detection was 2×10^6 cells/ml.

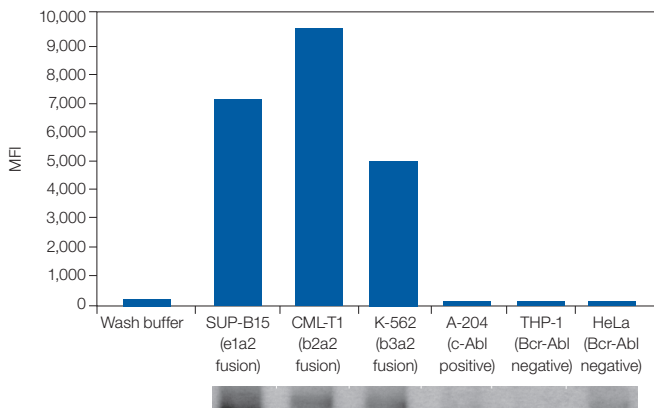


Fig. 5. Bio-Plex assay (top) and western blot (bottom) detection of three Bcr-Abl isoforms. The isoforms (see Figure 2) were detected in three CML cell lines and absent from three control cell lines.

Conclusions

Antibody pairs reactive with EGFR point mutation demonstrated suitable specificity for use in disease management and monitoring.

The Bio-Plex immunoassay for the detection of Bcr-Abl translocations was more sensitive than western blotting by about 4 orders of magnitude. The Bcr-Abl specific antibody pair recognized all three Bcr-Abl isoforms tested.

We 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 tissue samples.

Reference

Amann J et al., Aberrant epidermal growth factor receptor signaling and enhanced sensitivity to EGFR inhibitors in lung cancer, *Cancer Res* 65, 226–235 (2005)

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CST antibodies exclusively developed and validated for Bio-Plex phosphoprotein and total target assays.



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