The Detection of Phosphoproteins by Bio-Plex® Assays in Cell, Xenograft and Human Tumor Lysate.

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Abstract

The regulation of phosphorylation-dependent cell signaling plays an important role in the development of several human diseases, most notably cancer. Accordingly, many new cancer therapeutics specifically target phosphoproteins. Bio-Plex®, a multiplexable bead-based ELISA platform can be used to screen for levels of phosphoproteins. The objective of this study was to demonstrate the ability to detect phosphorylated and non- phosphorylated biomarker target, such as Erk in cell, xenograft and clinical human tissue lysates using Bio-Plex® assays. Using the Bio-Plex® assays, we determined there was a reduction of phosphorylated Erk in cell and xenograft samples treated with the receptor tyrosine kinase inhibitor Gefitinib. These results were validated using Western blots and IHC. In addition, changes in phospho-proteins could also be detected in human tissue samples. Decreased phospho-Erk was observed in tumor tissue compared to normal tissue. Our results demonstrate that results obtained by Bio-Plex® are comparable to conventional methods. Our goal is to use Bio-Plex® assays for the detection of cancer biomarkers in clinical samples.

Introduction

Mitogen-activated protein kinases (MAP Kinases), a family of serine/threonine kinases are activated by a broad range of extracellular signals which lead to the phosphorylation of threonine and tyrosine residues. Extracellular signal regulated kinase (Erk) is a subfamily of MAP kinases that plays a critical role regulating cellular proliferation and differentiation. Therefore, the phophorylation state of signal transducing proteins such as Erk are key determinants in the initiation and progression of cancer. Clinicians need to understand the degree of activation of a particular protein or pathway and the engagement with downstream components in order to identify the best treatment for each patient. Standard laboratory and clinical assays such as immunohistochemistry (IHC), ELISAs and Western blots are constrained as they can only detect expression of a limited number of proteins at once. In contrast, the Bio-Plex® (Luminex) bead-based ELISA platform has the potential to detect cells signaling events of up to 100 protein targets from a single sample. In a partnership, Cell Signaling Technology and Bio-Rad Laboratories developed, optimized and validated Bio-Plex® assays to detect and measure therapeutic targets and determinants of therapeutic efficacy. The objective of this study was to determine if the Bio-Plex® assay was equivalent or better than IHC and Western blot assays *in vitro* and *in vivo* using xenograft models and also using human clinical samples.

Methods

Lysate preparation

- For the cell lysate study, HCC827 cells (non-small cell lung cancer) were treated with 100ng/ml of EGF or inhibited with 1mM Gefitinib (Astra-Zeneca), then stimulated with EGF. Untreated cells were used as controls.
- For the xenograft study, 107 HCC827 cells were injected subcutaneously into Nude mice (Taconic).
  - Mice were administered either vehicle control (Tween-80) or 150 mg/kg of Gefitinib.
  - Tumors harvested 24 hour after treatment.
- Human lung tumor samples and normal adjacent tissue obtained from the Cooperative Human Tissue Network.
- 30mg of tissue was placed in Bio-Plex® cell lysis buffer, homogenized by mechanical lysis.
- Cells lysed with Bio-Plex® cell lysis buffer (Bio-Rad)
- Protein concentration measured.

Bio-Plex®

- Bio-Plex® kits for total Erk2 and phospho Erk 1/2 (Thr202/Tyr204, Thr185/Tyr187) were used to evaluate all lysates.
- Lysate (0.2mg/ml) was combined with Total or Phospho-Erk conjugated beads.
- Biotin-labeled detection antibodies added.
- Beads detected with fluorescently labeled strept-avidin.
- Samples analyzed with the Bio-Plex® Suspension Array System.

Immunohistochemistry and Immunocytochemistry

- Cell and tumor samples cut to 4 µm thickness.
- Antigen retrieval performed in 10 mM citrate/Tris buffered saline, pH 6.0.
- Slides washed then incubated in 3% hydrogen peroxide.
- Sections blocked in 5% normal goat serum for 1 hour.
- Slides incubated over night at 4°C with appropriate primary Ab.
- Anti-rabbit secondary antibody (Vector) added. Vector ABC Elite Kit reagent used for detection.

Cell Signaling TECHNOLOGY®

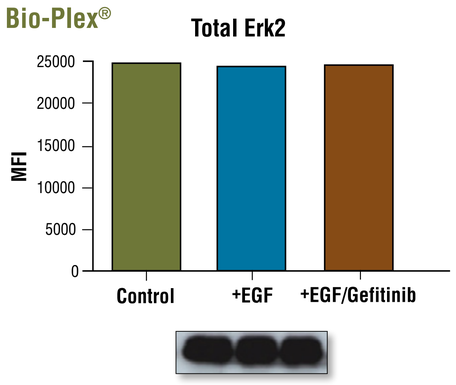
BIO-RAD *CST and Bio-Rad have an alliance in which CST's expertise is applied to screen and validate capture and detection antibody pairs to expedite Bio-Plex® assay development.*

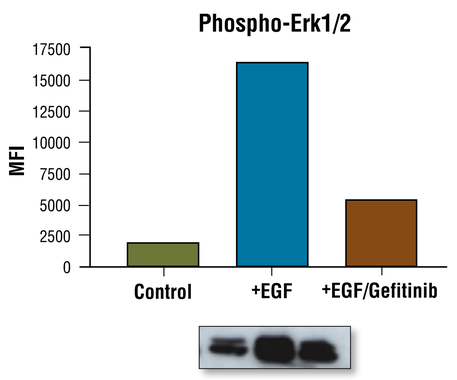
Results

CELL LYSATE

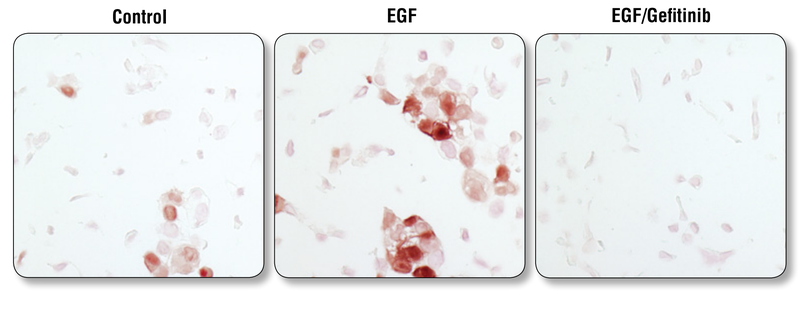
Erk

Bio-Plex®





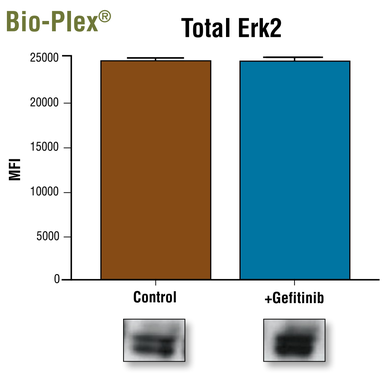
Phospho-Erk

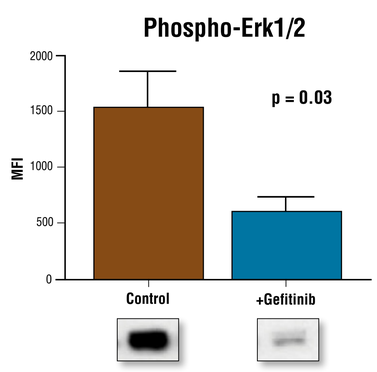


XENOGRAFT

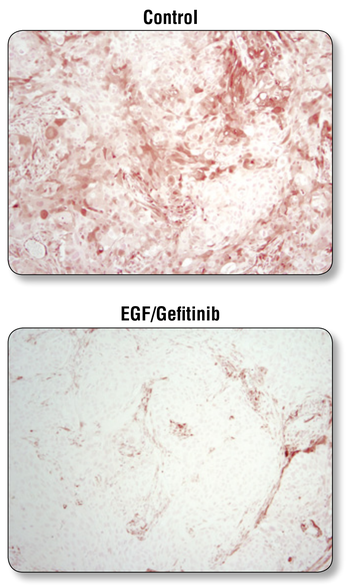
Erk

Bio-Plex®

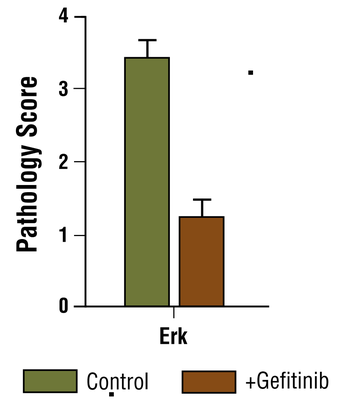




Phospho-Erk



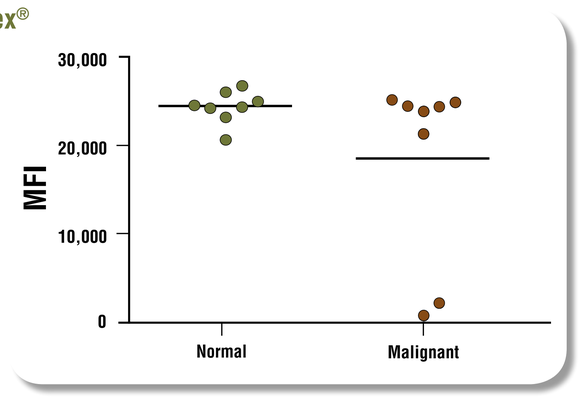
IHC Analysis

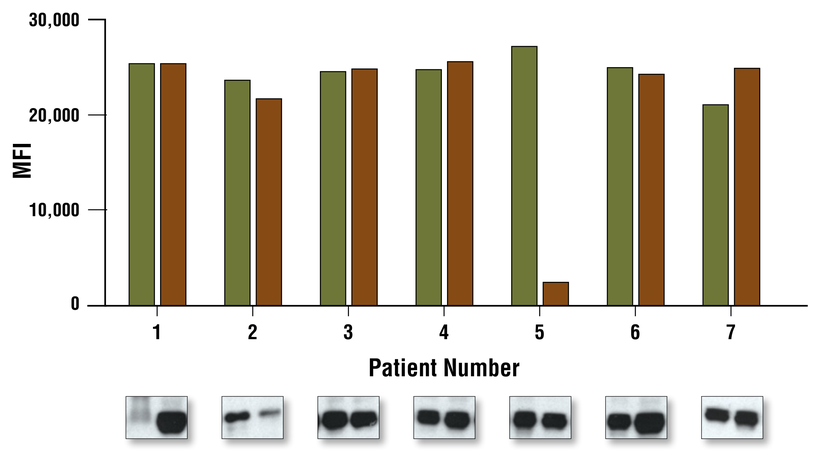


LUNG TISSUE

Erk

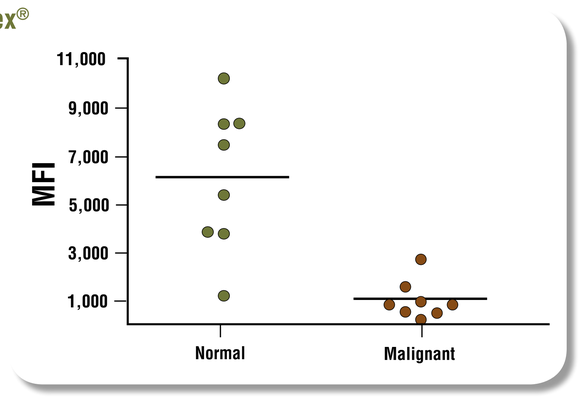
Bio-Plex®

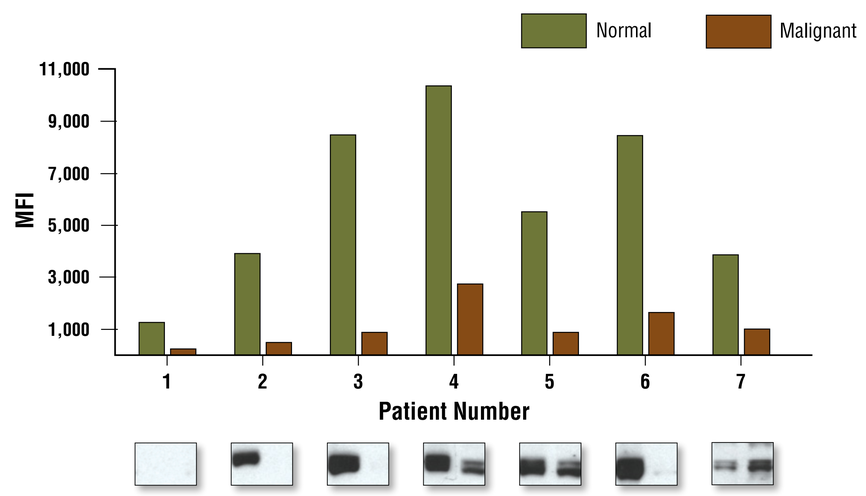




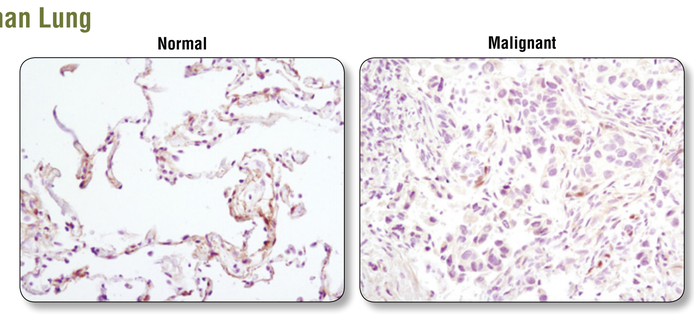
Phospho-Erk

Bio-Plex®





Human Lung



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Conclusions

- Gefitinib treatment resulted in decreased phosphorylation of Erk in both cell and xenograft samples as detected by Bio-Plex®, Western blot and ICC/IHC.
- The Bio-Plex® assay can be used to detect phospho-proteins from cell lysate xenograft and human tissue samples and suggest that the Bio-Plex® assay would be applicable for use in clinical samples.
- The Bio-Plex® assay offers a simple way to quantify proteomic biomarkers using small sample volumes from cell lysate and tumor tissue.

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