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 multiplex bead-based ELISA platform can be used to screen for biomarkers of cancer progression. The objective of this study was to demonstrate the ability to detect phospho-proteins from tissue lysates and cell lines using the Bio-Plex® assay. Using the Bio-Plex® assay, we determined there was a reduction of phospho-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 phosphorylation status of signal transduction proteins such as Erk are known to determine 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 a given time point. 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, HCC267 cells (non-small cell lung cancer) were treated with 100ng/ml of EGF or inhibited with Gefitinib (Astra-Zeneca), then stimulated with EGF. Untreated cells were used as controls.

- For the xenograft study, 10^7 HCC267 cells were injected subcutaneously into Nude mice (Taconic).

Lysate preparation

- Lysate preparation

- Bio-Plex® kits for total Erk and phospho Erk 1/2 (Thr202/Tyr204, Thr180/Tyr182) were used to detect all lysates.

- Lysate (35μg) was combined with Total or Phospho-Erk conjugated beads. Bio-Plex® beads were conjugated to capture and detection antibodies.

- Anti-rabbit secondary antibody (Vector) added.

- Samples analyzed with the Bio-Plex® Suspension Array System.

Immunohistochemistry and Immunocytochemistry

- Cell and tissue samples cut to 4 μm thickness.

- Antigen retrieval performed in 0.01M pH 6.0 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 overnight at 4°C with anti-Abs. (specific antibody).

- Anti-rabbit secondary antibody (Vector) added.

- Vector ARC Elite Kit reagent used for detection.

Conclusions

- Gefitinib treatment resulted in decreased phosphorylation of Erk in both cell and xenograft samples as detected by Bio-Plex®.

- The Bio-Plex® assay offers a simple way to quantify phospho-proteins from small sample volumes from cell lysate and tumor tissue samples.

- The Bio-Plex® assay offers a simple way to quantify phospho-proteins from small sample volumes from cell lysate and tumor tissue samples and suggest that the Bio-Plex® assay is suitable for use in clinical samples.

ACKNOWLEDGMENTS: We would like to thank Mike Lewis for animal work and Dr. Arlin Rogers for evaluating IHC samples.