Application of a nanoimmunoassay platform to assess changes in EGFR-dependent signaling pathways in lung cancer cell lines: surgical resections and laser-capture microdissection from patient-derived tumor cells exposed to EGFR tyrosine kinase inhibitors

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Introduction

To fully enable the vision of "bench-to-bedside" requires the development of not only novel therapies, but novel techniques for evaluating their efficacy in cell lines, animal models and primary tumor material. This report describes our development of a technique employing a nanoimmunoassay platform (Firefly ™) for the analysis of signaling protein activation in primary non-small lung cancer (NSCLC) solid tumors.

In our previous report, we assessed changes in phosphorylated and total ERK in lung cancer cell lines after exposure to erlotinib, a small molecule tyrosine kinase inhibitor (TKI) of the epidermal growth factor receptor (EGFR). Data from Firefly were compared to results obtained from standard western blotting using phosphorylated and total antibodies directed against ERK. We reported greater inhibition and more constitutively active ERK1 and ERK2 in TKI-sensitive NSCLC cells compared to TKI-resistant NSCLC cells.

Here we extend those findings from cell lines to tumor specimens obtained from patients. Previously, analysis of such samples at the protein activity level was prohibitive due to the small number of cells that can be practically collected with this method. In addition, we optimized a method for laser capture micodissection (LCM) using instrumentation that avoids the need for excessive drying of the specimens prior to LCM (mmi CellCut). We examined ERK activity in surgically resected NSCLC specimens and in a limited set of needle core biopsies collected from patients. These samples were stored as snap frozen specimens, sectioned while frozen, and tumor cells were isolated by LCM.

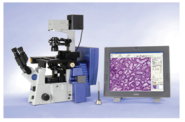
We measured ERK activity in core sections (12 um) and LCM isolated subpopulations of fewer than 500 cells. In 9 of 9 NSCLC specimens obtained at the time of surgical resection we were able to detect mono- and di-phosphorylated ERK isoforms along with non-phosphorylated ERK isoforms. In a small subset of patients that had core biopsies of the tumor prior to surgery, we could demonstrate measurements of ERK phosphorylation status. Duplicate specimens obtained from the same tumor showed excellent reproducibility of signal.

In conclusion, our study provides a demonstration of how this enabling technology can provide clinicians with clinically relevant metrics for evaluating key signaling proteins thus more fully realizing a "bench-to-bedside" relationship between research and clinical application. Application of this technology to tumor specimens obtained from patients on clinical trials with TKI may be of value.

Workflow and Assay

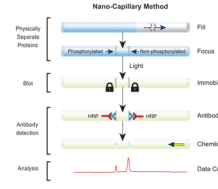
Core Biopsy (During Surgical Resection)

Core Biopsy (Prior to Surgery)



MMI LCM Technology (Wet cut 500 cells)

Cell Biosciences Nano-Immunoassay (200 nl volume)



The Cell Biosciences Assay

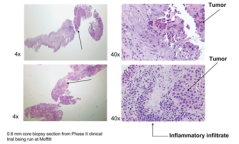
Figure 1. Cell Biosciences nano-immunoassay:

- **Fill:** A 5 cm capillary is filled with a separation buffer containing sample proteins and fluorescent peptide standards.
- **Focus:** Isoelectric Focusing (IEF) is used to focus the proteins.
- **Immobilize:** The focused proteins are immobilized by exposing the capillary to the UV light source.
- **Antibody:** Labeled antibodies are flowed through the capillary to bind to the immobilized proteins.
- **Detection:** Whole capillary imaging is performed using chemiluminescence.
- **Data Collection:** Capillary images are converted to signal vs. pI graphs for analysis.

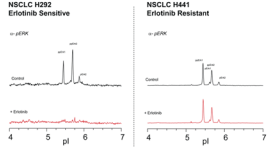
Cell Models to Patient Samples

The Problem:

Heterogeneity of primary tissue: only a percentage of material is NSCLC

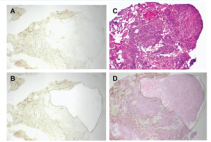


Cell lines give binary results because of their homogeneity



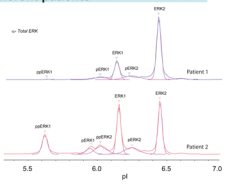
The Solution:

Laser Capture Microdissection of patient NCSLC biopsies



Punch biopsy from patient with NSCLC. (A) Primary tissue section prior to LCM. (B) Same section after removal of tumor-enriched material. (C) Adjacent serial section stained with H&E. (D) Overlay of stained and LCM serial sections to verify section purity for tumor cells.

Activity of ERK isoforms varies in different patients



Conclusions

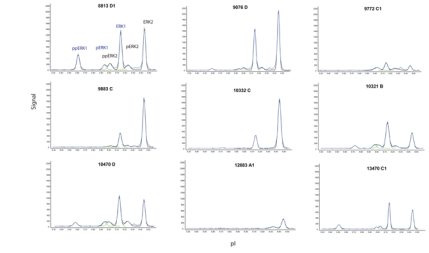
80% of tumors have evidence of pERK; higher than reported in lung cancer using IHC (approximately 35% - S. Vincent, British Journal of Cancer, 2004)

We have demonstrated the ability to measure signaling proteins in tumor cores that have few tumor cells (tumor cellularity < 10%)

This method makes possible the analysis of signal transduction pathways in samples previously inaccessible

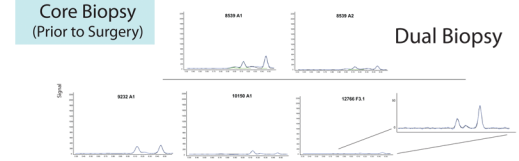
Results

Core Biopsy (During Surgical Resection)

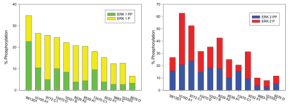


Core Biopsy (Prior to Surgery)

Dual Biopsy



Quantitation



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