The Simple Western Rapidly Generates Quantitative Profiles of MAPK and PI3K Proteins in Clinical Specimens

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Abstract

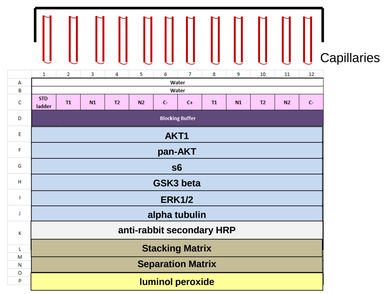
In this era of targeted therapeutics, there is a need for highly sensitive and quantitative methods to measure proteins in clinical specimens to define and predict of specific therapies for patients. However, the current gold standard in proteomic analysis, Western Immuno Blotting, has many manual steps, is insensitive and provides only semi-quantitative data. Here, we present the first report of a novel automated instrument "Sally^{TM}," a nanovolume size-based protein separation platform used to quantify proteomic profiles of clinical specimens. Since the instrument automates all steps of proteomic analysis including sample loading, size-based protein separation, immunoprobing, washing, detection and data quantification, we are able to make up to 96 measurements in a single experiment, in a highly quantitative manner, minimizing errors due to manual variability. We were able to quantify proteins in either surgical specimens preserved at -80C in Optimum Cutting Temperature Compound (OCT) or fine needle aspirates flash-frozen at time of collection. Tissues were homogenized and lysed in a commercially available Bicine/CHAPS lysis buffer, denatured and loaded in duplicate in a 384-well plate at a final concentration of 0.2-2 mg/ml in each well. 40 nanoliters of lysate was used for each protein measurement. We measured 10 proteins (including AKT and ERK) from the MAPK signaling pathways, normalized to loading controls (β-actin or tubulin). It took 1 hour to prepare a run and 14 hours of unattended automated machine time to generate the analyzed data. Therefore this technology enables us to report highly quantified protein profiles to the clinical team in less than 24 hours of receiving a clinical sample. In the course of 1 week, we quantified ERK1 and ERK2 in over 100 clinical specimens and in a subset of 22, measured an additional panel of proteins including PI3K proteins [AKT1, AKT2, pan-AKT, GSK3b, S6]. The ability to make rapid and quantitative measurements will be useful to measure and predict responses to targeted therapeutics. The Sally platform is an extremely efficient, quantitative and high-throughput platform that can be used to rapidly generate proteomic profiles for clinical specimens for the development of novel diagnostic and predictive biomarkers.

Goals

- Develop nano-proteomic assays to analyze MAPK and PI3K protein profiles in clinical specimens
- Determine sensitivity and feasibility of using the new 384-well instrument instrument for clinical specimens
- Analyze paired tumor and adjacent normal tissue from patients

Methods

Schematic of 384-well format assay



Results

Loading nanogram amounts of total protein gives linear quantification

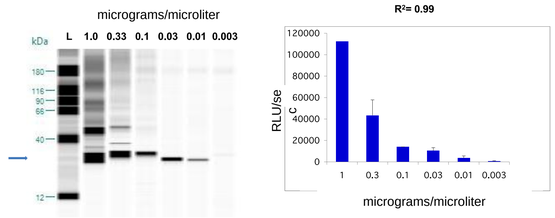


Figure 1. PRDX protein was measured in serial dilutions of Hela lysate. Final concentration per well is indicated, in 5 microliters total volume per well. Similar results were obtained for ERK , pERK, and AKT.

Results

TKI-sensitive vs resistant cells can be distinguished by p-ERK1 and GSK3b levels

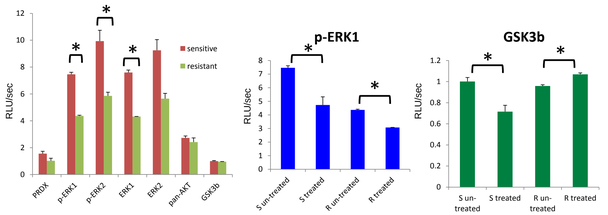


Figure 2. HCC827 cells were treated with gefitinib in vitro. * p= <0.05

ERK1 and ERK2 show a wide range of baseline levels in 100 clinical specimens (log_{10} scale)

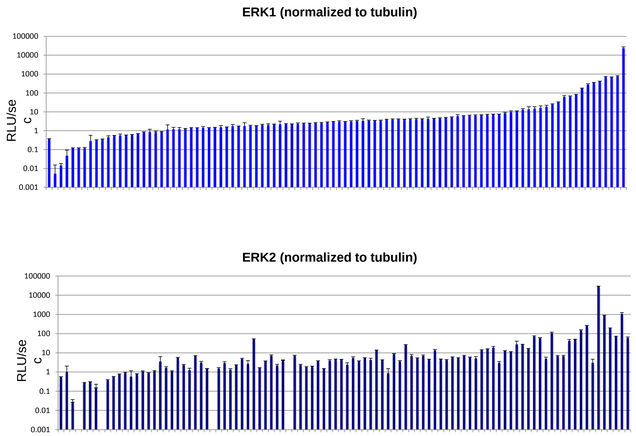


Figure 3. ERK1 and ERK2 were analyzed in 100 frozen tumor and normal tissue lysates in a series of 5 over-night runs.

Results

PI3K pathway was profiled in 22 FNA's and tissue samples in two overnight runs

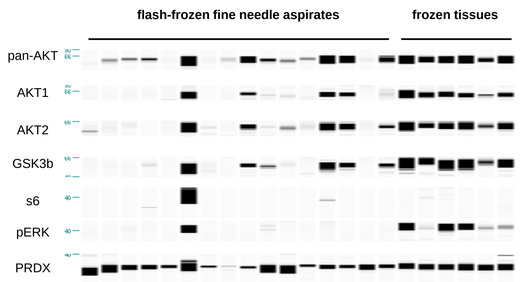


Figure 4. PI3K profiles for 22 FNA and tissue samples were generated in two overnight runs (11 samples per run, 1 hour set-up time and 14 hours unattended instrument time per run).

Summary

We have used a novel 384-well format Simple Western (Sally^{TM}) to analyze protein expression of oncogenic signaling proteins in normal and tumor clinical specimens.

The assay is very sensitive: proteins could be detected when nanograms of total protein were loaded into each well.

Baseline levels of signaling proteins vary over several logs across specimens.

Protein profiles are rapidly generated, with results returned in 6-24 hours, depending on the number of proteins analyzed.

Nanovolume size-based protein analysis can be used to develop biomarkers for cancer diagnostics and monitoring of therapeutic response.