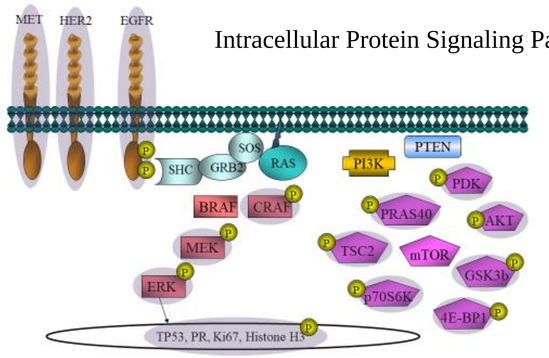
Analytical Validation of a Quantitative Intracellular Protein Signaling Panel for the Analysis of FFPE Breast Cancer Biopsies

Jinho Lee, Todd Camp, Janice Patterson, Gordon B. Mills and Christopher L. Corless
Knight Diagnostic Laboratories and Knight Cancer Institute, Oregon Health & Science University, Portland, OR

Introduction

Measuring protein expression by immunohistochemistry (IHC) in formalin-fixed, paraffin-embedded (FFPE) tissue is routine in clinical labs. However, current assays are limited to just 1 to 3 biomarkers per slide and histochemical signals are only semi-quantitative. Recently, a multiplexed panel of oligonucleotide-tagged antibodies has been developed (Nanostring 3D Vantage Solid Tumor Panel), comprised of 27 antibodies, including 13 phosphorylated protein targets, specifically designed to interrogate the MAPK and PI3K/mTOR signaling pathways (ref.1). The panel uses the Nanostring nCounter system to quantitate oligonucleotides that are released by UV light after antibody binding. We undertook a validation of this panel with the goal of creating a new clinical assay for core biopsies of solid tumors.



Intracellular Protein Signaling Panel

Methods

Four micron sections of formalin-fixed paraffin-embedded (FFPE) cancer cell lines (controls) or 18-21 gauge cores of solid tumors (fixed within 3 minutes of biopsy) were subjected to citrate-based antigen retrieval and incubated overnight with the cocktail of oligo-tagged antibodies. After washing, the oligo-tags were released by UV light and quantitated on the Nanostring nCounter system. A set of 6 FFPE cancer cell lines were selected as positive controls and included on every run to assess antibody performance and support inter-run normalizations. 28 metastatic breast cancer core biopsies were analyzed to serve as a comparative cohort.

Results

- In FFPE cancer cell lines detection of progesterone receptor (high in MCF-7, BT474, T47D) and HER2 (high in HCC1954, SKBr3, BT474) matched reverse-phase protein array data. Pre-incubation of MDA-MB-468 with EGF led to expected increases in p-EGFR, p-MEK1/2, p-ERK1/2, p-AKT and p-S6.
- Among 10 rapidly-fixed FFPE solid tumors, signals for EFGR, p-EGFR, HER2, TP53, TSC2 and p-S6 were all consistent with known genomic alterations.
- There was good correlation with IHC results for PR and HER2 on sections of FFPE breast cancer core biopsies (**Fig.1**).
- Slide pre-treatment with lambda phosphatase eliminated phospho-protein signals (**Fig.2**).
- A bioinformatics strategy was developed to allow comparison of samples between runs (**Fig 3**).
- Analysis of 6 FFPE cancer cell lines selected as positive controls showed excellent inter-run reproducibility ($r \geq 0.92$).
- Data from 28 metastatic breast carcinoma core biopsies were generated to serve as a reference cohort (**Fig.4**).

Figure 1 - Correlation With IHC

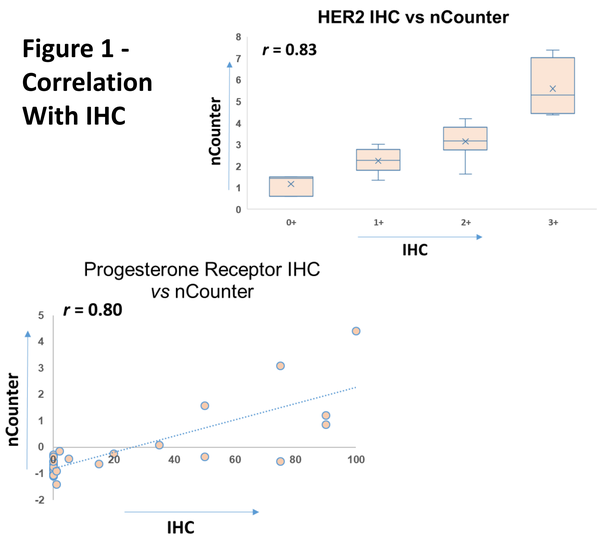


Figure 2 – Specificity of Phosphoprotein Abs

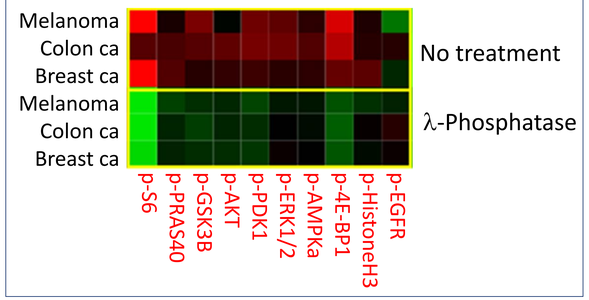
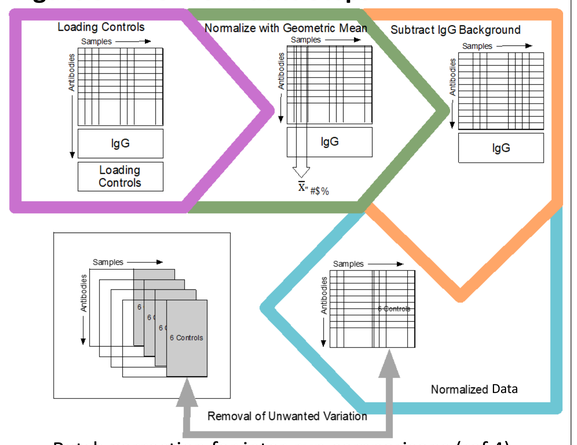
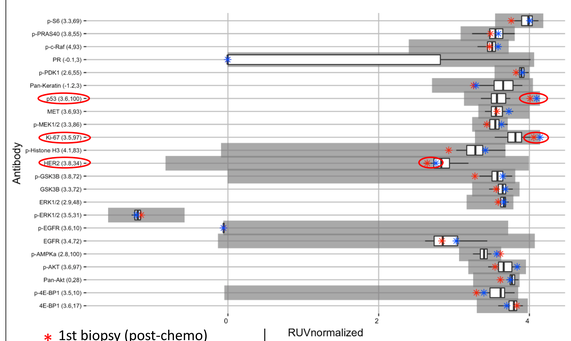


Figure 3 – Bioinformatics Steps



Batch correction for inter-run comparisons (ref.4)

Figure 4 – Comparison of 2 serial biopsies to cohort of 28 metastatic breast cancers



Conclusions

The 3D Vantage Solid Tumor Panel provides a robust, quantitative approach to measuring 27 cell signaling biomarkers from a single section of FFPE tissue in <48 hours. The panel is useful for investigating treatment-induced changes in tumor cell signaling, but there are some limitations: 1) specimens must be fixed within minutes of biopsy to avoid protein de-phosphorylation (ref.2); 2) the assay requires a minimum of 40% tumor fraction; 3) any normal tissue should be excluded. The excellent performance of this slide-based approach suggests that it can be adapted to the new GeoMx Digital Spatial Pathology system (Nanostring), which uses micro-mirrors to focus UV light and release tags only from selected cells of interest. With the appropriate controls and bioinformatics steps, quantitative protein profiling using oligo-tagged antibodies holds great promise for future assays.

References

1. Lee J. Geiss GK, Demirkan G, Vellano CP, Filanoski B, Lu Y, Ju Z5 Yu S, Guo H, Bogatzki Ly, Carter W, Meredith RK, Kirshnamurthy S, Ding Z4, Deechem JM, Mills GB. Implementation of a Multiplex and Quantitative Proteomics Platform for Assessing Protein Lysates Using DNA Barcoded Antibodies. Mol Cell Proteomics. 2018 Jun;17(6):1245-1258.
2. Leal MF, Haynes BP, MavNeill FA, Dodson A, Dowsett M. Comparison of protein expression between formalin-fixed core cut biopsies and surgical excision specimens using a novel multiplex approach. Breast Cancer Res Treat. 2019 Jun;175(2):317-326
3. Peloquin JM, Goel G, Kong L, Huang H, Haritunians T, Sartor RB, et al. Characterization of candidate genes in inflammatory bowel disease–associated risk loci. JCI Insight. 2016 Aug 18;1(13):e87899.
4. Molania R, Gagnon-Bartsch JA, Dobrovic A, Speed TP. A new normalization for the Nanostring nCounter gene expression assay. bioRxiv [Internet]. 2018 Jul 23 http://biorxiv.org/lookup/doi/10.1101/374173