APPLICATION NOTE

Correlate Protein Expression with Genomic Data and IHC

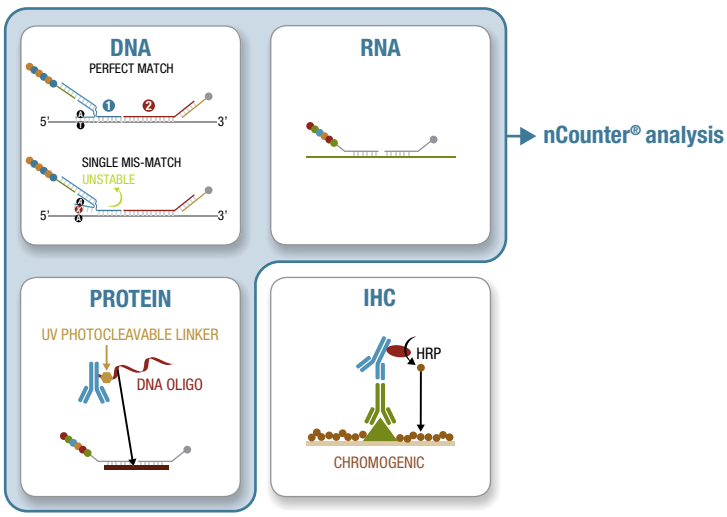
NanoString® + CST Inside: Measure Protein, RNA and DNA in FFPE Tumor Specimens

Introduction

Initiation and progression of tumors are marked by oncogenic mutations and changes in transcript (RNA) abundance, but are ultimately carried out by proteins. Correlation between RNA and protein abundances can vary by gene and by tissue (1, 2). Immunohistochemistry (IHC) of formalin-fixed, paraffin embedded (FFPE) clinical tumor samples reveals information about protein expression in the context of tissue architecture, but chromogenic IHC readout is semi-quantitative. Sample profiling with multiplexed, quantitative readouts for protein, DNA, and RNA is desirable in order to maximize information yielded from limited tissue samples and to obtain a broader understanding of tumor evolution. However, this can be challenging due to variations in sample handling and fixation protocols, and quantitative comparison between protein, RNA, and mutations in the same sample is lacking. In this tech note, we demonstrate multi-analyte characterization of non-small cell lung cancer (NSCLC) FFPE samples. IHC-validated, NanoString®-barcoded antibodies were used to compare abundance of key signaling proteins, in parallel with analysis of RNA transcript abundance and DNA single nucleotide variant (SNV) mutations on the NanoString® 3D Biology™ and nCounter® platform. We present results demonstrating concordance of RNA/protein quantification with clinical gold standard IHC methodology.

Methods

The Vantage 3D™ DNA:RNA:Protein Solid Tumor Assay was designed to cover key solid tumor driver mutations and common signaling pathways for integrated genotype and phenotype analysis of minimal FFPE sample. DNA coverage includes somatic variants associated with lung adenocarcinoma, thyroid carcinoma, pancreatic adenocarcinoma, colorectal adenocarcinoma, and melanoma relevant to new and existing clinical trials, drug pipelines, tumor subtypes, and drug resistance and response. 770 cancer-related genes and >100 single nucleotide variants (SNV) from the same FFPE NSCLC specimen were analyzed on the nCounter® Platform. FFPE NSCLC specimens were simultaneously stained with a panel of NanoString®-barcoded antibodies from CST specific to a variety of cancer-relevant proteins for 3D Biology™ quantification, and adjacent tissue sections were used for IHC validation.

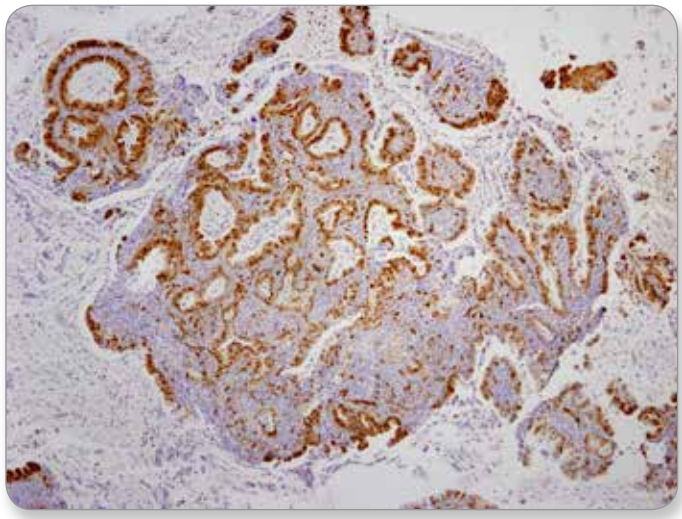


nCounter® analysis

Briefly, RNA and DNA were extracted using the Qiagen AllPrep® kit according to manufacturer's recommendations. Purified DNA was quantified by Qubit fluorometric assay. For the The Vantage 3D™ DNA:RNA:Protein Solid Tumor Assay, exactly 5 ng sample DNA was used as input in the SNV amplification reaction. An additional FFPE slide was deparaffinized and rehydrated, then subjected to antigen retrieval prior to incubation with NanoString oligo-labeled antibodies according to the nCounter® Vantage 3D Protein FFPE protocol. Oligos were photocleaved with UV illumination for 3 min and collected for hybridization. Hybridization and quantification of DNA and RNA:Protein (cleaved oligos) were carried out according to NanoString protocols on the nCounter® platform. The resulting raw data were analyzed using NanoString's nSolver™ Software and Advanced Analysis package.

Immunohistochemistry

FFPE slides were deparaffinized and rehydrated, then subjected to antigen retrieval in 10 mM citrate or 1 mM EDTA in a microwave. After blocking, primary antibodies diluted in SignalStain® Antibody Diluent #8112 were applied to the slides and were incubated O/N at 4°C. After washing, SignalStain® Boost IHC Detection Reagent #8114 (rabbit) or #8125 (mouse) was applied for 30 min, then slides were exposed to SignalStain® DAB Chromogen #8059. Slides were counterstained with hematoxylin then coverslipped with SignalStain® Mounting Medium #14177. The following antibodies were used for IHC: Met (D1C2) XP® Rabbit mAb #8198, EGF Receptor (D38B1) XP® Rabbit mAb #4267, Ki-67 (8D5) Mouse mAb #9449, Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370, Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb #4858, and Phospho-PRAS40 (Thr246) (D4D2) XP® Rabbit mAb #13175.

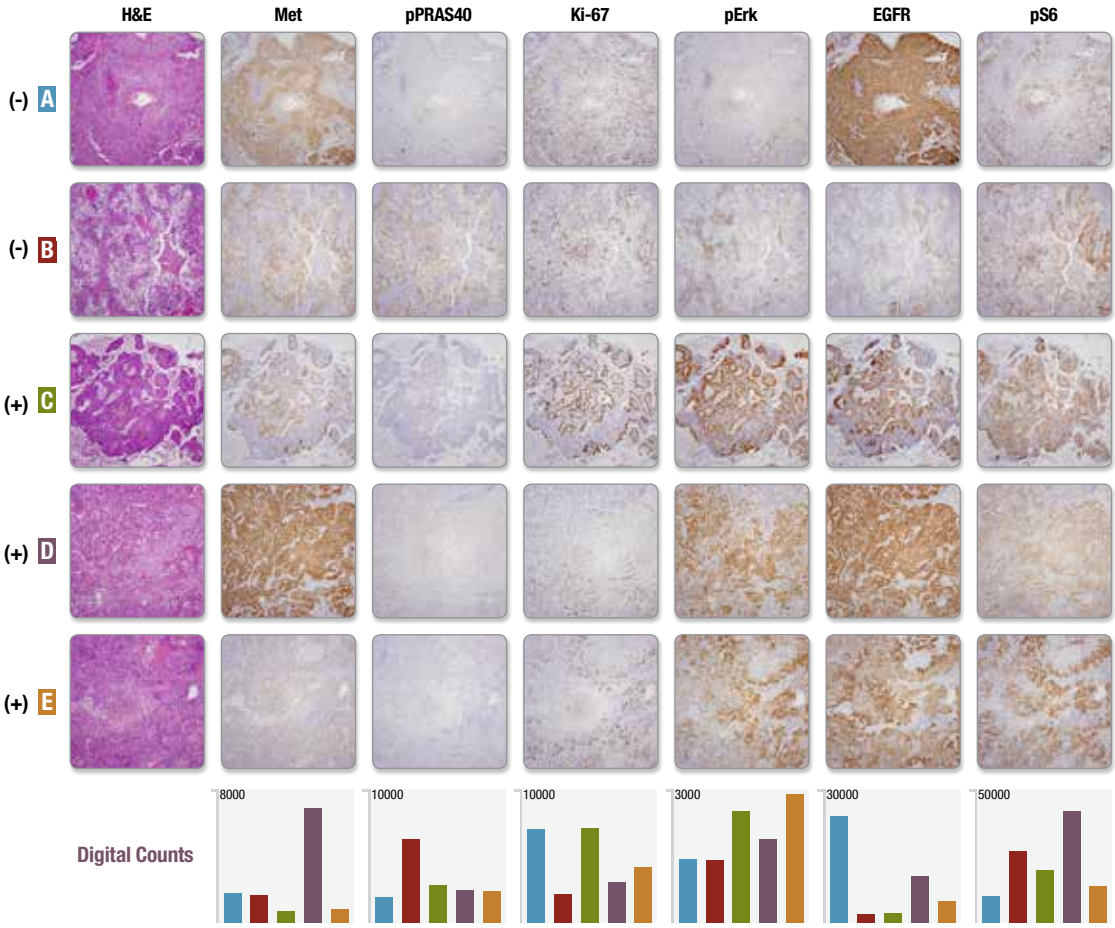


Immunohistochemical analysis of paraffin-embedded human non-small cell lung carcinoma using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370.

Correlate Protein Expression with Genomic Data and IHC

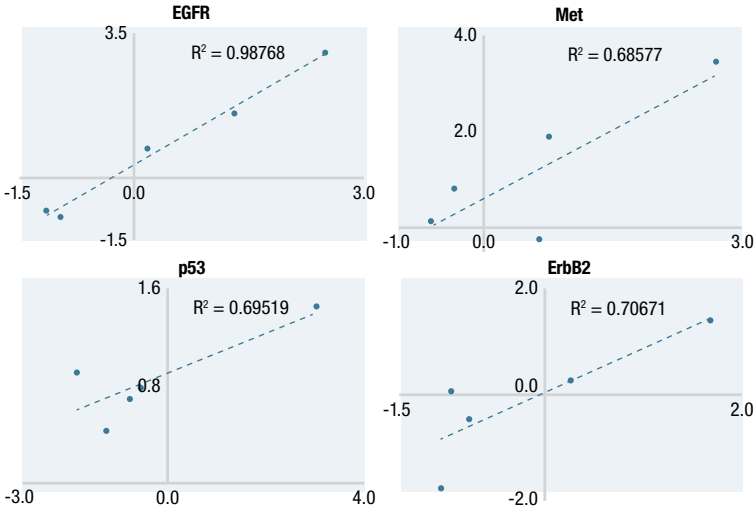
Results

Concordance of IHC and nCounter® analysis of protein expression using validated CST antibodies



Serial sections of each NSCLC specimen were stained with H&E and with antibodies for each of the noted proteins. Tumor Ras G12C genotype of each sample (A–E) obtained from SNV analysis is indicated (+/-) on the left. An additional single serial section was simultaneously stained with DNA-barcoded antibodies. Normalized digital counts from the nCounter® analysis for each protein are shown from left to right, color-coded to each sample (A–E).

Correlation of RNA and protein levels



Expression of RNA and protein for each individual sample is plotted. Values represent the ratio of expression in the individual sample to the geomean of the expression across all of the samples. Protein expression is represented on the Y-axis and gene expression is represented on the X-axis.

Conclusions

- Frictionless integration of IHC-validated antibodies from CST with 3D Biology™ Technology enables efficient analysis of key cancer pathways from as little as two FFPE sections for genotype and phenotype information.
- Quantifying protein abundance in addition to RNA/DNA analysis is crucial, as correlation between protein and RNA abundances can vary (1, 2).
- Concordance was demonstrated between nCounter® digital counts of protein abundance and gold standard IHC methods using IHC-validated antibodies from CST.
- The power of integrated genomic and proteomic analysis is demonstrated by the association of the detected Ras mutations and the corresponding changes in Erk1/2 protein phosphorylation.

References

1. Schwanhäusser B et al. (2011) *Nature* 473(7347):337-42.
2. Kosti I et al. (2016) *Sci Rep* 6:24799.

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