Lung Cancer Profiling with 3D Biology™ Technology

Introduction

The global cancer burden is growing at an alarming rate,[1] and lung cancer is still the most deadly cancer-related disease.[2] However, the five-year relative survival rate of lung cancer patients is slowly rising due to improvements in treatment and novel therapies.[1] In particular, personalized pharmacotherapy has gained traction in clinical oncology. Therefore, understanding the molecular mechanisms of disease and identifying new biomarkers for lung cancer are imperative.[2] Identification of targetable driver mutations and genomic rearrangements, which affect genes involved in cell proliferation and survival, has been a major influence in lung cancer treatment.[3]

As an example, the anaplastic leukemia kinase (ALK) gene fusions/reciprocal translocations occur in up to 5% of non-small cell lung carcinoma (NSCLC). These rearrangements are associated with a high response rate of these patients to the ALK inhibitor crizotinib[4] showing improved efficacy over chemotherapy.[3] However, ALK FISH assays are challenging due to technical difficulties requiring repeat testing, borderline cut-off values, false positive results, and false negative results.[5] Key mutations have also been found to be associated with treatment response, such as EGFR and KRAS.[6,7] Studies profiling key mutations and gene fusions in addition to other analytes can yield novel insights about the molecular mechanisms that drive disease pathogenesis.[7]

Despite advances in personalized pharmacotherapy, the challenging problem of obtaining adequate tumor tissues for molecular analysis in clinical practice remains. Small biopsies involve taking a piece of diseased tissue for pathologic examination to obtain a preoperative diagnosis or optimal treatment decision. For these small biopsy specimens, simple and rapid analysis methods with high sensitivity and specificity are needed.[1]

NanoString's 3D Biology™ technology enables simultaneous analysis of DNA, RNA, and protein simultaneously on the NanoString nCounter® system for research use. Here, we demonstrate this capability through the simultaneous detection of Echinoderm microtubule-associated protein like-4 (EML4) and ALK fusion gene expression, oncogene SNV, and intracellular and intranuclear MAP kinase pathway protein activation from minimal lung cancer FFPE samples.

Material & Methods

In this this study, 16 lung FFPE samples were profiled according to the workflow shown in Figure 1.

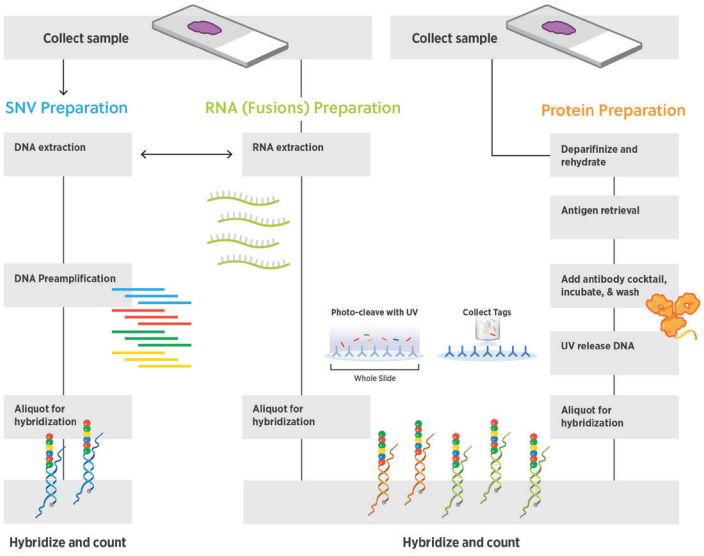
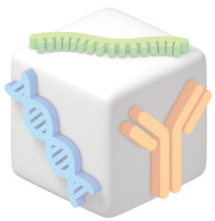


Figure 1 nCounter® Vantage 3D™ FFPE workflow

Briefly, extraction of RNA and DNA were performed using the Qiagen AllPrep kit according to manufacturer's recommendations. An additional slide was incubated with NanoString barcoded antibodies according to the Vantage 3D™ Protein FFPE protocol. Hybridization and quantification of DNA, RNA, and protein were carried out according to NanoString protocols on the nCounter platform. The resulting raw data were analyzed using NanoString's nSolver® Advanced Analysis.



Results

SNV and Fusion Transcript Detection

Mutational data from NGS were available for the 16 FFPE lung samples profiled in this study. Using the Vantage 3D DNA SNV Solid Tumor and Lung Fusion Panel, we successfully profiled all 16 lung FFPE samples simultaneously for the presence of known cancer-related SNV and RNA fusions from 5 ng of gDNA and 150 ng RNA (Figure 2).

An EGFR (L858R) SNV and multiple KRAS variants (G12C, G12V, G12D, G13C, and G13D) at the exon 2 hotspot were correctly detected in two and nine samples, respectively. In addition to the SNV confirmed in our cohort, we simultaneously confirmed four of 16 samples were positive for the EML4-ALK fusion gene. Our SNV and fusion transcript detection demonstrated high accuracy and specificity when compared to existing data.

Protein Detection

After successful determination of mutational status, these samples were analyzed using the Vantage 3D Protein Solid Tumor Panel, to measure the expression of 26 targets using antigen-specific antibodies directed against protein or phospho-protein epitopes. The antigens include 13 cell-proliferation-associated proteins. As demonstrated in Figure 3, protein detection shows high reproducibility regardless of quantitation using a protein-only assay format versus multi-analyte assay formats for simultaneous analysis of RNA and protein or DNA, RNA, and protein.

Looking at a subset of samples carrying a range of the confirmed mutations, we see significant activation of the cell proliferation activating RAS-RAF-MEK-ERK signaling pathway. This is evident by an increase in protein phosphorylation of the MEK and ERK kinases. This was particularly pronounced in samples with KRAS mutations, a known activating mutation (Figure 4). A similar activation was observed in samples expressing the AML-ALK fusion transcript (Figure 5). Those specimens with oncogenic EGFR variants showed a more moderate increase in the phosphorylation of ERK MAPK signaling pathway proteins (Figure 6). In all cases, no change was observed in total ERK protein expression.

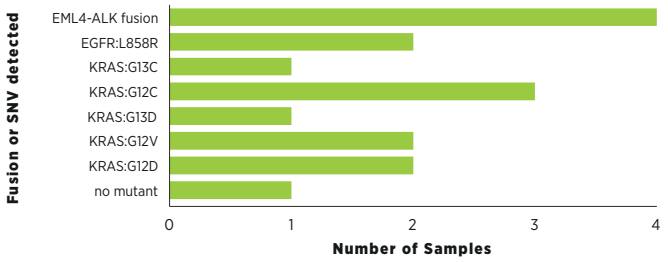


Figure 2 SNV and fusion accurately detected in 16 lung samples with known SNV and fusion status.

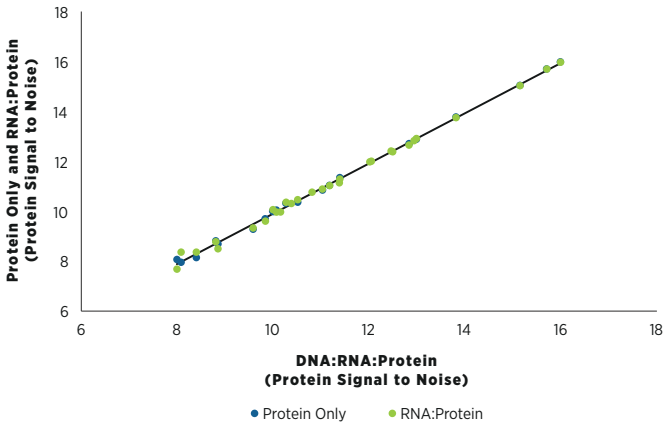


Figure 3 Protein quantification shows high correlation on FFPE samples whether run alone, with RNA, or with DNA:Fusion:Protein all combined on a single lane for simultaneous analysis on the nCounter platform.

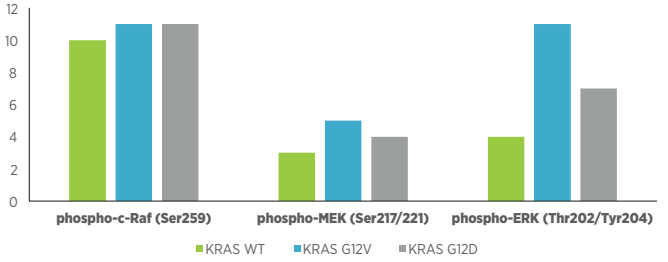


Figure 4 Compared to the KRAS WT sample, samples with the KRAS G12V and G12D SNV shows increased ERK MAPK pathway activation, as demonstrated by an increase in RAF, MEK, and ERK phosphorylation.

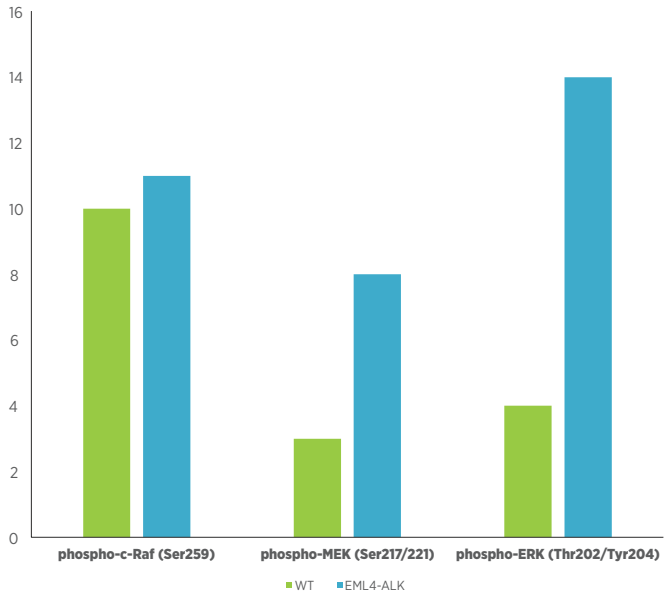


Figure 5 Compared to the WT sample, the fusion-positive sample shows increased ERK MAPK pathway activation, as demonstrated by an increase in RAF, MEK, and ERK phosphorylation.

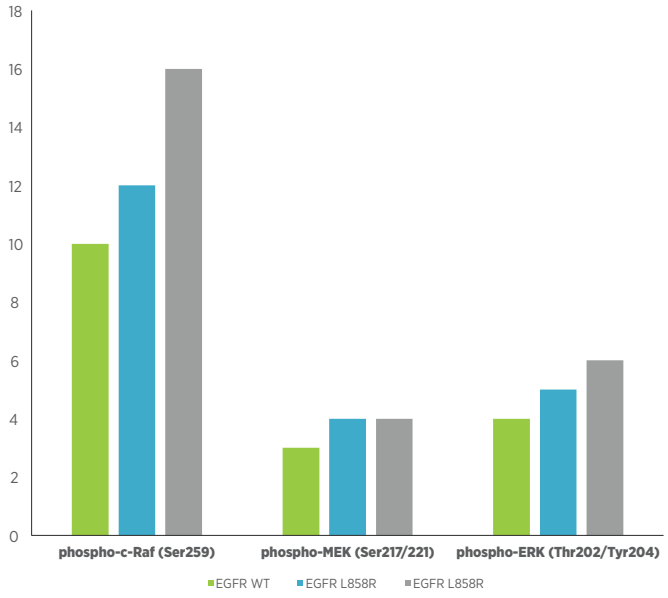


Figure 6 Compared to the WT sample, the EGFR L858R positive sample show increased ERK MAPK pathway activation, as demonstrated by an increase in RAF, MEK, and ERK phosphorylation.

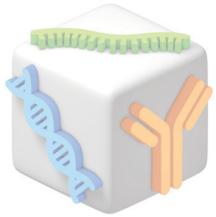
Discussion

We present here the rapid, simultaneous quantitation of SNV, fusion transcripts, and total and phospho-proteins from lung FFPE sections. This proof-of-principle for 3D Biology technology demonstrates the power of simultaneous identification of the mutational load for cancer-associated SNV and fusion genes and the direct impact on the activation of proteins in the proliferation-associated MAP kinase signaling pathway, all from two FFPE slices.

Notably, we demonstrated reliable detection of reciprocal translocations as shown for the fusion of EML4 and ALK. This event represents a distinct type of mutation that may drive the development of NSCLC. The fusion protein been shown to be highly oncogenic and ultimately results in cancer development.[8] Several clinical trials have demonstrated the remarkable efficacy of crizotinib for the treatment of metastatic NSCLC in patients who harbor ALK rearrangements.[1] Furthermore, we here report the identification of activating variants in oncogenes with simulations mechanistic confirmation through protein profiling. Similar profiles have been reported previously for EGFR and KRAS mutations in NSCLC.[9]

Similarly, an increase in MAPK activation is associated with cancer progression and metastasis. Successful inhibition of elevated activated proteins in the MAPK axis may lead to reduction in tumor growth.[7]

In summary, our approach eliminates the need for large sample input and use of multiple specialized methods and instrumentation for the quantitation of genetic variants (genotyping array, NGS), chromosomal translocations (FISH, NGS), and protein activation (mass spectrometry, western blot, RPPA). Furthermore, it removes the need to harmonize data from different platforms and reduces the time from sample to result. Our 3D Biology approach provides a holistic view of biology from minimal sample tissue.



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