Molecular Pathology Abstracts, 6/17

Editors: Donna E. Hansel, MD, PhD, chief, Division of Anatomic Pathology, and professor, Department of Pathology, University of California, San Diego; John A. Thorson, MD, PhD, associate professor of pathology, director of the Clinical Genomics Laboratory, Center for Advanced Laboratory Medicine, UCSD; Sarah S. Murray, PhD, professor, Department of Pathology, and director of genomic technologies, Center for Advanced Laboratory Medicine, UCSD; and James Solomon, MD, PhD, resident, Department of Pathology, UCSD.

Whole genome single-cell copy number profiling on FFPE tissue samples

Single-cell genomic methods take the concept of analyzing intratumor genetic heterogeneity to its logical conclusion. Traditionally, however, single-cell methods can only be used to analyze fresh or rapidly frozen tissue because formalin fixation and paraffin embedding degrades tumor DNA and cross-links proteins. The authors described and validated a novel technique for performing single-cell whole genome copy number analysis on formalin-fixed, paraffin-embedded (FFPE) tissue. For their study, FFPE tissue blocks were cut into 100-µm-thick sections for microdissection. The tissue was then processed to remove cross-links, treated with enzyme to break down extracellular material, and passed through a fine needle to release nuclei. Next, the nuclei were sorted by flow cytometry to separate single nuclei into individual wells. Once sorted, single nuclei were treated with a cocktail of DNA repair enzymes, and whole genome amplification was performed using a previously reported method shown to work well with poor-quality fragmented DNA. After library preparation, sequencing was performed using Illumina multiplex sequencing, and copy number analysis across the entire genome to a resolution of approximately 700 kb was determined. To compare this technique to those that use rapidly frozen tissue, the authors performed a pilot study in which FFPE and frozen tissue sections were each microdissected and processed as appropriate for each tissue type. The single-cell copy number analyses were compared to each other, as well as to copy number analysis of a FFPE section of bulk tumor that contained about 100,000 cells. Overall, the copy number analyses, both quantitatively and qualitatively, for each of the specimens were highly concordant. The authors showed that the DNA repair step was necessary when using FFPE tissue to significantly improve the whole genome amplification step, as artifacts and significant variability were otherwise seen. A control experiment, where frozen tissue underwent the DNA repair step, showed that no artifacts were introduced. The authors next demonstrated the biologic utility of this groundbreaking technique in a few proof-of-concept experiments. For example, they were able to compare subpopulations of tumor cells in ductal carcinoma in situ (DCIS) versus adjacent invasive cancer in two cases. In one case, the subpopulations of tumor cells seen in the DCIS were distinct from those seen in the invasive components, suggesting that intratumoral heterogeneity developed early in the course of disease, with one of the clones later acquiring alterations that led to invasion. In the other case, the DCIS and invasive components contained similar subpopulations of cells, suggesting that the ability to invade was acquired early in tumor development followed by subsequent intratumoral heterogeneity. Overall, this study presents an exciting method for examining copy number variants in FFPE tissue and demonstrates the importance of a critical DNA repair step. Among the drawbacks are that the integrity of the initial DNA affects the effectiveness of the assay and that the whole genome amplification method, which allows for complete coverage of the genome, is not particularly suited for detecting point mutations and small deletions. Yet the possibility of performing single-cell genomic studies using FFPE tissue presents enormous research and clinical opportunities.

Martelotto LG, Baslan T, Kendall J, et al. Whole-genome single-cell copy number profiling from formalin-fixed paraffin-embedded samples. *Nat Med*. 2017;23(3):376–385.

Correspondence: Dr. Jorge S. Reis-Filho at reisfilj@mskcc.org, or Dr. Britta Weigelt at weigeltb@mskcc.org, or Dr. James B. Hicks at jameshic@usc.edu

Intratumor heterogeneity and tumor evolution of non-small cell lung cancer

While several recent studies have characterized the molecular attributes of primary lung adenocarcinomas and squamous cell carcinomas, data on intratumor heterogeneity are limited. In an ongoing multi-center prospective cohort study in the United Kingdom, titled Tracking Non-Small Cell Lung Cancer Evolution through Therapy, or TRACERx, researchers will be following the lung tumors from 842 non-small cell lung cancer (NSCLC) patients to understand their evolution. Molecular and genetic techniques will be used to analyze the spatial and temporal heterogeneity of NSCLC. The authors reported on data from the first 100 patients recruited into the TRACERx study, focusing on spatial intratumor heterogeneity of NSCLC with respect to mutations and copy number alterations. They collected primary tumor samples from 100 patients, with disease stages ranging from IA through IIIA, all of whom had not received systemic therapy. The authors took samples from at least two tumor regions separated by 0.3 to 1.0 cm and performed whole exome sequencing on 327 tumor regions and 100 matched germline samples to a median depth of 426×. They observed extensive intratumor heterogeneity, with 30 percent of somatic mutations and 48 percent of copy number alterations classified as subclonal. The individual tumors, however, exhibited a wide variability in tumor heterogeneity, with the number of subclonal mutations ranging from two to 2,310. The authors observed a correlation between the proportion of subclonal copy number alterations and tumor stage. Additionally, the patients with higher proportions of subclonal copy number alterations had reduced relapse-free survival. This association remained significant in a multivariate analysis that accounted for age, smoking history, histology, adjuvant therapy, and tumor stage. Interestingly, a static measure of chromosome disruption did not correlate with survival, suggesting that the rate of ongoing dynamic chromosomal instability, rather than the state of the genome, is prognostic. For each individual tumor, the authors mapped the evolutionary history, identifying the individual subclones and creating a phylogenetic tree. Overall, they identified 525 mutation clusters, with a median of five per tumor. By analyzing the individual tumors, the authors were able to describe the evolutionary pressure acting on the lung tumors. For example, some targetable driver mutations, including those in *EGFR, MET*, and *BRAF*, were almost exclusively clonal and, therefore, deemed to be early events in tumorigenesis, while it was suggested that the subclonal driver mutations, including those in *PIK3CA* and *NF1*, were later events in tumor evolution. In some tumors, subclonal genes were seen only in a single region, highlighting concerns about limited tumor sampling. Therefore, classifying the driver mutations as clonal or subclonal has obvious implications for choosing effective targeted therapy. Overall, this study highlights the importance of understanding intratumor heterogeneity and demonstrates that chromosome instability, as measured by increased intratumor heterogeneity of copy number alterations, is a significant adverse prognostic factor.

Jamal-Hanjani M, Wilson GA, McGranahan N, et al. Tracking the evolution of non-small-cell lung cancer. *N Engl J Med.* 2017. doi:10.1056/NEJMoa1616288.

Correspondence: Dr. Charles Swanton at charles.swanton@crick.ac.uk