Spatially resolved, multiplexed digital characterization of protein abundance in FFPE tissue sections: application in preclinical mouse models

Abstract

Background:
Characterization of the abundance, distribution, and colocalization of key immunoregulatory proteins within the tumor microenvironment is necessary for a thorough understanding of tumor immune responses. Historically, immunohistochemistry and immunofluorescence have been used to assess spatial heterogeneity of proteins in tissue slices, but these techniques are of limited utility due to the challenge of measuring multiple targets in parallel. We recently developed a platform to enable spatially-resolved protein detection with the potential to simultaneously quantify up to 800 targets from a single formalin-fixed paraffin-embedded (FFPE) sample slide, termed Digital Spatial Profiling (DSP). To demonstrate preclinical applications of DSP, we have developed an assay to detect and quantify ~20 key immune- oncology (IO) targets in mouse FFPE tissue sections.

Methods:
DSP uses DNA oligo tags covalently linked to detection reagents (primary antibodies) via a UV photolabile linker to identify targets in situ and enable quantitation via the standard nCounter® platform. A slide-mounted FFPE tissue section is incubated with a cocktail of oligo-labeled primary antibodies, and a serial section is stained with low-plex visible/fluorescent probes (e.g., nuclear staining probes, or select antibody pairs such as anti-CD3) to generate an image of the FFPE tissue slice morphology. Regions of interest (ROIs) in the tissue/tumor are then identified and sequentially illuminated with UV light to release the DNA-oligos. Following UV illumination, the photoexcited oligos are released into the aqueous layer above the tissue slice, collected via microcapillary aspiration, and stored in an individual well of a microtiter plate. Oligos are then hybridized to nCounter optical barcodes to permit ex-situ digital counting of as many as 800 different analytes localized within a single ROI, which can be referenced using image capture software.

Results:
We demonstrate preclinical applications of this technology by characterization of a panel of immune proteins on mouse FFPE tumor and normal tissue sections. We demonstrate that this approach enables protein detection at single cell resolution, and enables simultaneous multiplexed detection of AKT, B7-H3, Beta-Catenin, CD3, CD4, CD45, F4/80, PD-L1, Ki67, pan-cytokeratin, STAT3 and additional key IO targets.

Conclusions:
The ability to measure DNA, RNA, and protein at up-to 800-plex from single slices of FFPE tissue may improve the early evaluation of drug targets with high-resolution spatial information, enable the discovery of key immune biomarkers in mouse tissue (tumors and inflammation et al), and accelerate the preclinical development of immunotherapies.

Conventional IHC

Spatially resolved, multiplexed protein characterization on FFPE

Highly Multiplexed Molecular Profiling with Optical Barcodes

Overall Workflow

Spatially resolved, multiplexed protein characterization on FFPE

Conclusions

- New application of NanoString barcoding enables multiplexed, digital, spatially-resolved protein profiling in FFPE mouse tissue
- Variable ROI selection permits profiling of 500 µm diameter areas down to single cells
- Detection capability to spatially resolve and multiplex up to 800 different proteins and RNAs on fixed tissue
- In situ multiplexed protein profiling in tumor microenvironment may enable novel biological insights and accelerate drug development