Visualization of Prostate Cancer Tissue Heterogeneity Using in situ Protein Multiplexing

An innovative multiplexing method to detect proteins

Qing Li, Christopher Sevinsky, Sean Dinn, Zhengyu Pang, Brion Sarachon, Megan Rothney, Bruce Colligan, Larry Douglass, Julia Carter, Jeremy Graff and Fiona Ginty

1GE Global Research Center Biosciences Organization, Molecular Imaging and Diagnostics Advanced Technologies Program; 2Lilly Research Labs, Eli Lilly and Company, Indianapolis, Indiana
3Wood Hudson Cancer Research Laboratory, Newport, Kentucky

Abstract

Immunohistochemistry (IHC) has become an increasingly important technique not only for assessing diagnosis in anatomical pathology, but also in drug development, biomarker discovery and translational research. However, traditional IHC only allows simultaneous detection of 3 to 4 proteins on the same tissue section, and its semi-quantitative results are subjective. Although proteomic profiling methods are high-throughput, the cellular and spatial content around them is lost. To meet the unmet need, we have developed a novel multiplexing-based sequential multiplexing and automated imaging, data analysis technology platform that allows high-throughput protein profiling in situ on a single paraffin-embedded tissue section. This technology platform will allow integrated multiple protein expression analysis at the subcellular level segmented using cellular compartment markers. This platform will be a paradigm shift that allows pathologists to see the molecular interactions and activated signal pathways hidden behind the tissue morphology. In one clinical study using prostate FFPE tissue based Biomarker imaging and discovery platform, we’ve developed a novel technology platform for high-throughput, the cellular and spatial expression analysis at the subcellular level segmented using cellular compartment markers. This technology platform allows for the simultaneous detection of multiple proteins of interest in a single tissue section. The sequential multiplexing method requires antibodies directly labeled with either Cy3 or Cy5 dyes, NHS-ester mediated conjugation has been used in our process. All primary Antibodies from different vendors against total of 28 selected targets were first validated using primary/secondary IF staining and then conjugated with Cy3 or Cy5 dyes. Dye protein ratio was in the range of 2-6 dyes per AB for all targets. Free dyes was removed by desalting column chromatography. Direct conjugated Antibodies will be re-validated again using the same tissue in the primary validation. Pan-cytokeratin was used for segmentation between epithelial and stroma regions. Na+K+ATPase S6 and DAPI as cellular compartment markers were used for single cell segmentation of cell membrane, cytoplasm and nuclei respectively. A designated pathologist selected regions of interest (ROI) for the whole study. ROI of each slide covers heterogeneity from normal prostate to cancerous tissue with various Gleason grades. Approximately 25 to 45 images per slice based on tissue availability and quality. 2 distinct labeled antibodies at each round of staining, total of 16 sequential multiplexing steps for all targets was achieved. All acquired images will be analyzed through an automated image analysis algorithm developed in house. The biomarker result and clinical data will be analyzed by our biostatistians.

Materials and Methods

- 10x archived FFPE prostate tissue sections from surgical resected specimen of 74 patients at St. Elizabeth Medical Center between 1986 to 1990 were used for the study.
- The sequential multiplexing method requires antibodies directly labeled with either Cy3 or Cy5 dyes, NHS-ester mediated conjugation has been used in our process.
- All primary Antibodies from different vendors against total of 28 selected targets were first validated using primary/secondary IF staining and then conjugated with Cy3 or Cy5 dyes. Dye protein ratio was in the range of 2-6 dyes per AB for all targets.
- Direct conjugated Antibodies will be re-validated again using the same tissues in the primary validation.
- Pan-cytokeratin was used for segmentation between epithelial and stroma regions.
- Na+K+ATPase, S6 and DAPI as cellular compartment markers were used for single cell segmentation of cell membrane, cytoplasm and nuclei respectively.
- A designated pathologist selected regions of interest (ROI) for the whole study. ROI of each slide covers heterogeneity from normal prostate to cancerous tissue with various Gleason grades. Approximately 25 to 45 images per slice based on tissue availability and quality.
- 2 distinct labeled antibodies at each round of staining, total of 16 sequential multiplexing steps for all targets was achieved.
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Results

- Representative multiplexed images from the same field.
- Segmentation between epithelial and stroma regions.
- Single cells and compartments are detected, thousands of data points are generated for each cell – 10^6 cells/patient.

Conclusion

- This novel FFPE tissue based multiplexing technology platform will be an invaluable tool for in situ multivariate analysis of proteins.
- Using this technology, multiple pathways involved in cancer progression can be investigated simultaneously.
- It allows spatial denotation of multiplexed biomarkers in situ and generating molecular map on the top of tissue morphology.
- The visualization of molecular map combined with virtual traditional H&E morphology provide an unique and powerful tool for development of diagnostic and predictive tests.

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