Imaging in cancer immunology: Phenotyping of multiple immune cell subsets in-situ in FFPE tissue sections

B. Wendik¹, J.R. Mansfield², C.C. Hoyt², E. Stack², M. Feldmann³, C.B. Bifulco⁴, B. Fox⁵
¹Quantitative Pathology, PerkinElmer LAS (Germany) GmbH, Rodgau, Germany
²Quantitative Pathology, PerkinElmer, Hopkinton, MA, USA
³Surgical Pathology, University of Pennsylvania- CRB, Philadelphia, PA, USA
⁴Pathology - Hematology, Providence Cancer Center, Portland, OR, USA
⁵Molecular and Tumor Immunology, Providence Cancer Center, Portland, OR, USA

Aim: There has been a rapid growth in the field of tumor immunobiology as a result of recent successes in cancer immunotherapies, and it is clear that immune cells play many sometimes conflicting roles in the tumor microenvironment. However, obtaining phenotypic information about the various immune cells in and around the tumor has been a challenge.

Methods: Tonsil and breast cases were labeled for CD4, CD8, CD20 and CK utilizing TSA and the Opal™ multiplexing method. Samples were imaged on the Vectra™ multispectral slide analysis system. Tissue pattern recognition and cell phenotyping as cytotoxic T cells, helper T cells, Tregs, and B cells, were made with inForm™ software.

Results: Existing methods can deliver phenotypic information on homogenous samples (e.g., flow cytometry) or morphologic information in single stain IHC. We present here a methodology for delivering quantitative per-cell marker expression and phenotyping, analogous to that obtained from flow cytometry, but from cells imaged in situ in FFPE tissue sections. This methodology combines: the sequential multi-marker labeling of up to 6 antigens using antibodies of the same species; automated multispectral imaging to remove autofluorescence and correct cross-talk between channels; automated image analysis that can quantitate the per-cell marker expression, cellular phenotyping, counting cells separately in the tumor stromacomartment, and provide x,y coordinate data from which spatial distance calculations can be made. We show a 6-plex assay in breast cancer showing the multiplexed staining, per-cell quantitation and cellular phenotyping in FFPE tissue sections, as well as methods to explore the spatial distributions of the phenotyped cells in and around the tumor.

Conclusions: Multispectral imaging allows immune cell phenotypes to be visualized and quantified simultaneously in the same tissue section enabling further study of the relationships and spatial distribution of these cells within the tumor microenvironment. This technology will enable improved understanding of the immune infiltrate in breast tumors thereby facilitating the rational design and use of immunotherapeutic agents in combination with standard systemic therapies.

Disclosure: B. Wendik, J.R. Mansfield, C.C. Hoyt and E. Stack: Employees of PerkinElmer. All other authors have declared no conflicts of interest.