Abstract Control Number: 2509
Poster Board Number: 8
Permanent Abstract Number: 3863

Ultivue
Profiling Cancer Biology

Development of specific multiplexed immunofluorescence immune assays to study mouse models of tumorigenesis

REACTION BIOLOGY

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Background

Immunotherapy has transformed the treatment of metastatic and recurrent solid tumors. Advances in technology in the past few years have created unprecedented opportunities to identify biomarkers of disease processes, especially by using multi-omics technologies and datasets to derive valid and useful signatures of disease. The use of pre-clinical mouse tumor models are widely used tools to demonstrate efficacy of novel immunotherapies. Despite their widespread use, a comprehensive view of their tumor-immune compositions and their relevance to human tumors has only begun to emerge. The use of specific mouse tissue phenotyping and multiplex immunofluorescence (mIF) assays offer the unique advantage of preserving the architectural features of the tumor and revealing the spatial relationships between tumor cells and immune cells. The urgency to discover and implement new biomarkers lays bare the need to integrate a variety of advanced pre-clinical tools to probe the dynamic nature of events occurring in the tumor immune microenvironment (TiME).

Methods

To investigate the microenvironment within murine tumor types we have utilized both normal spleen from a BALB/c mouse and two syngeneic mouse FFPE tissues(tumor indications 4T1, Breast and B16-F10, Melanoma, implanted into the mammary fat pad by SubQperior™ method, Reaction Biology, Freiberg, Germany). Optimized murine specific InSituPlex® (ISP) technology (Figure 1) was used to perform both single-plex DAB and multiplex immune profiling on mouse FFPE tumor serial sections. Alternating serial sections were stained with a cocktail of four primary antibodies (CD3, CD4, CD8 and FOXP3) or a single primary antibody, in parallel using an autostainer. Slides were imaged on the Zeiss Axio Scan Z1 providing high-quality images of the four targets in a single workday. The images were exported for downstream analysis with IndicaLabs HALOv3.1 software. Concordance of the singleplex DAB assay to the 4-plex mouse specific ISP assay was assessed qualitatively. Precision of the 4-plex assay was determined by quantifying the percent difference in cellular density and signal intensity of immune cell subtypes.

Ultivue staining technology

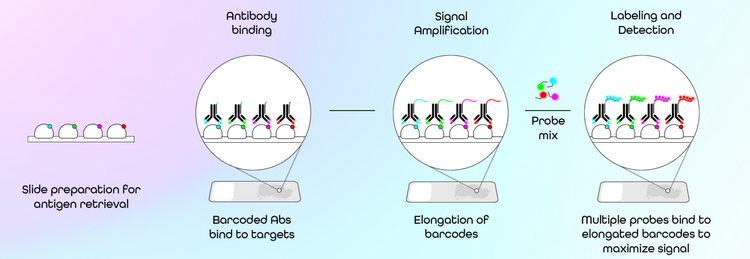

Figure 1. Overview of InSituPlex chemistry to develop multiplexed mouse panels

Results

Qualitative assessment and quantitative analysis from whole slide imaging revealed a high level of reproducibility of the assay, with each single markers' coefficients of variation falling within an acceptable range (range is <30 CV, Figure 2a-b). Each single marker testing verification showed an expected level of expression and expected staining pattern. The multiplex assay was highly concordant to the DAB singleplex assay (Figure 2d). Unique phenotypes from whole slide analysis followed the expected pattern, as indicated by the tissue and target type (Figure 2c, 3c, & 4c).

Assay Reproducibility

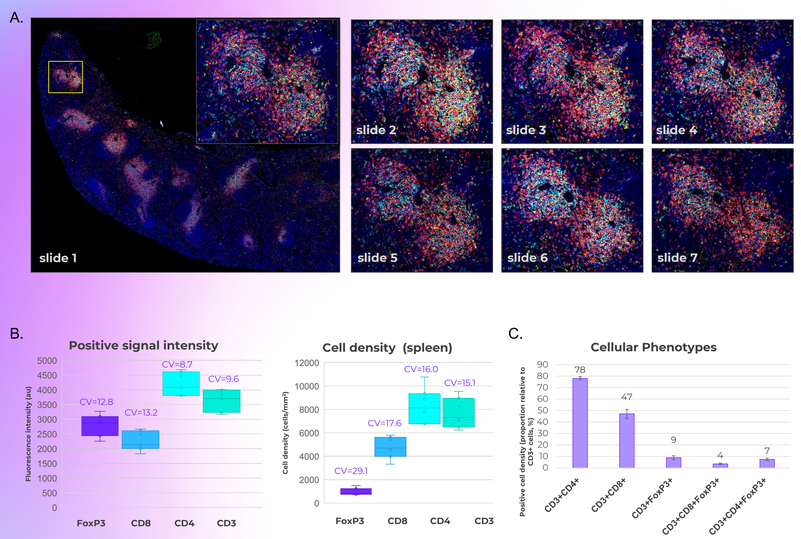

Figure 2. A. Mouse spleen intra run concordance. Shown are zoomed in regions from whole slide images (WSI) of seven serial sections stained with multiplex ISP. Qualitative analysis showed a high level of reproducibility in all 4 markers. **B**. Box Plot analysis of intensity and cell density for each marker in the seven serial sections. **C.** Bar graph showing the % of double or triple positive cells, relative to total CD3+ cells.

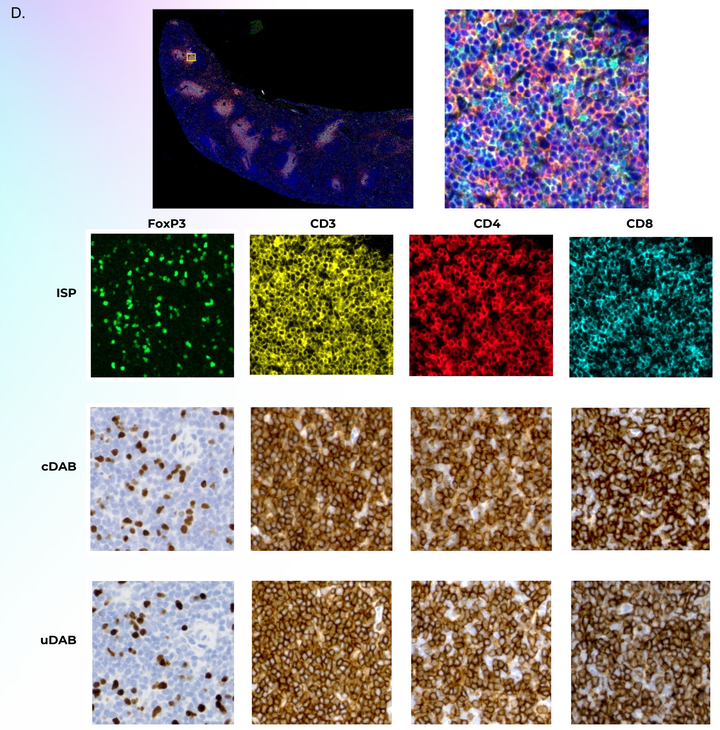

Figure 2. D. Multiplex image of mouse spleen. The whole slide and zoomed in region. Top panel single channel ISP, middle panel conjugate DAB, bottom panel unbarcoded DAB. Qualitative assessment of predicate comparison results reveals a high level of concordance between the DAB and ISP staining for each marker of the panel.

Verification in B16 whole slide melanoma tissue

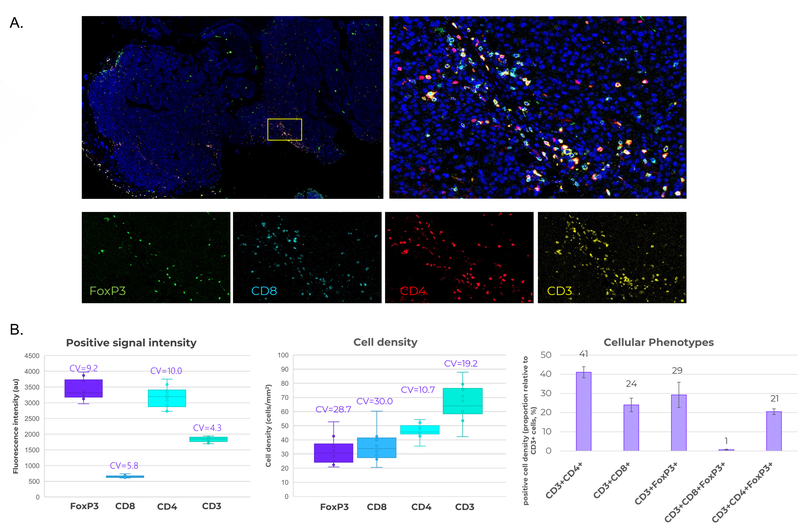

Figure 3. Validation in a syngeneic model of Melanoma A. Multiplex image staining of B16 melanoma. The whole slide and zoomed in region. **B**. Box Plot analysis of intensity and cell density for each marker in the seven serial sections. **C.** Bar graph showing the % of double or triple positive cells, relative to total CD3+ cells.

Verification in 4T1 whole slide breast tissue

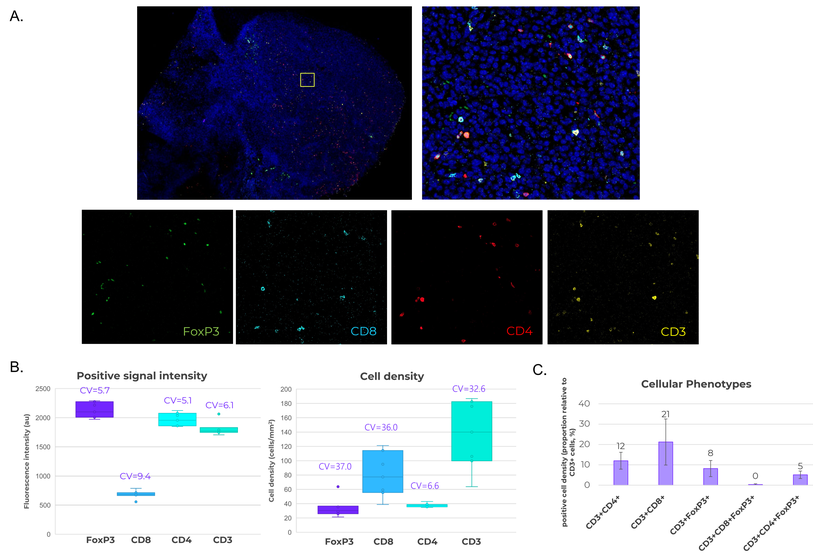

Figure 4. Validation in a syngeneic model of breast cancer A. Multiplex image staining of 4T1 breast. The whole slide and zoomed in region. **B**. Box Plot analysis of intensity and cell density for each marker in the seven serial sections. **C.** Bar graph showing the % of double or triple positive cells, relative to total CD3+ cells.

Conclusion

Our unique streamlined pre-optimized protocol enables mIF staining on specific mouse tissue to provide an improved workflow to investigate the immune system, including the analysis of the tumor immune microenvironment and mechanisms of action of immune-related drugs in preclinical syngeneic or GEMM mouse models.