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Cross-platform same slide multi-omics reveals Treg heterogeneity and links to spatial niche variability in pre-cancerous colonic inflammation

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Introduction

FOXP3+ regulatory T cells (Tregs) maintain immune tolerance, and their dysfunction contributes to autoimmunity, chronic inflammation, and cancer. In ulcerative colitis, Treg heterogeneity is well documented and linked to colon cancer. However, Treg states are typically defined by transcriptional profiles, which may be confounded by discordance between RNA and protein levels.

Here, we use same-slide multi-omics with CellScape™ precise spatial proteomics and CosMx® Spatial Molecular Imager to quantify FOXP3 protein alongside gene expression. We find that proteomics reveals distinct FOXP3 protein levels defining Treg states within different spatial neighborhoods and functional contexts, which are further confirmed by spatial transcriptomics.

Methods

Eight FFPE intestinal biopsies from a pre-cancerous condition (ulcerative colitis) were profiled with CellScape™ (34-plex VistaPlex), followed by CosMx® Human Universal Cell Characterization Panel (1K-plex) on the same slide. Whole-slide multimodal alignment used shared morphology markers and WsiReg to generate affine registrations. Segmentation masks were harmonized for pixel-level correspondence, enabling direct integration of protein and RNA per cell. CD4+ T cells were classified into $FOXP3_{low}$, $FOXP3_{mid}$, $FOXP3_{high}$ groups via quantitative automatic cell typing. Spatial neighborhoods (k=15) yielded 25 FOXP3-defined niches, and both protein and RNA features were included in neighborhood and state enrichment analyses.

Same slide multi-omics captures protein and RNA from a single tissue section

Fig. 1 Same-slide CellScape (34-plex VistaPlex, protein) and CosMx (1K-plex, RNA) profiling on FFPE tissue enables direct, high-fidelity, molecular integration, eliminating the need for serial sections.

Shared nuclear and morphology markers enable pixel-level CellScape-CosMx registration

Pixel-level alignment enables shared:
- Cell Segmentation
- Cell Typing
- Protein Expression

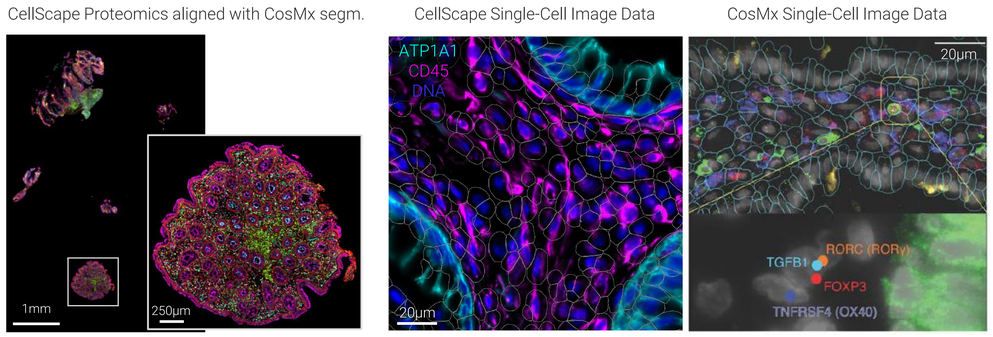


Fig. 2 Whole-slide affine registration via WsiReg using shared morphology markers

CellScape captures quantitative FOXP3 expression validated by same-tissue HCR™ Gold RNA-FISH

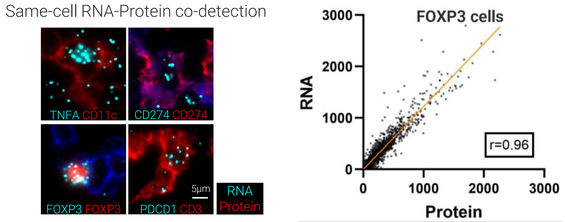


Fig. 3 Per-cell FOXP3 protein expression correlates strongly with HCR™ Gold RNA-FISH transcript abundance (r=0.96), validating quantitative CellScape protein measurements.

Quantitative Treg Stratification into $FOXP3_{low}$, $FOXP3_{mid}$, and $FOXP3_{high}$ phenotyping

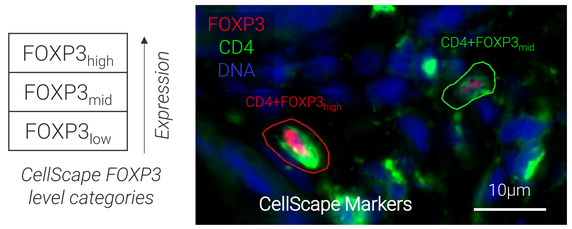
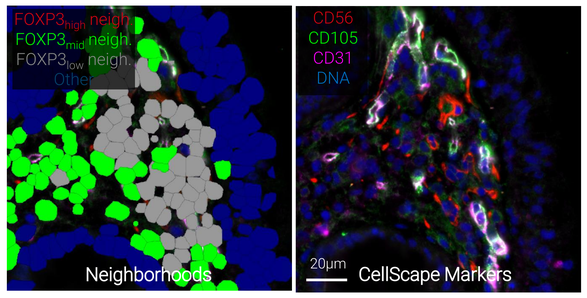
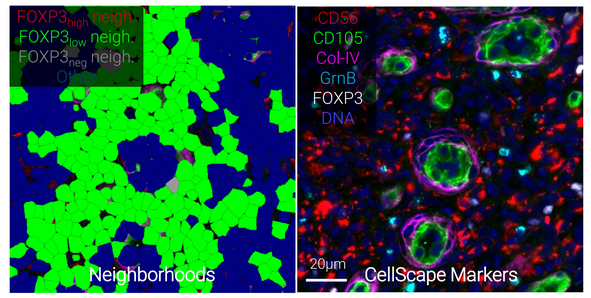


Fig. 4 Co-expression of nuclear FOXP3 and cytoplasmic CD4 CellScape protein expression at single-cell resolution stratifies Treg populations into three quantitative phenotypic tiers.

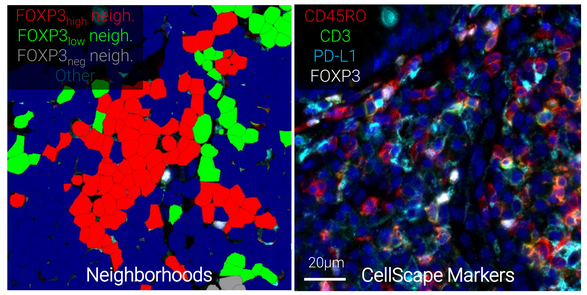
$FOXP3_{low}$ niches are enriched with stromal-vascular endothelial architecture



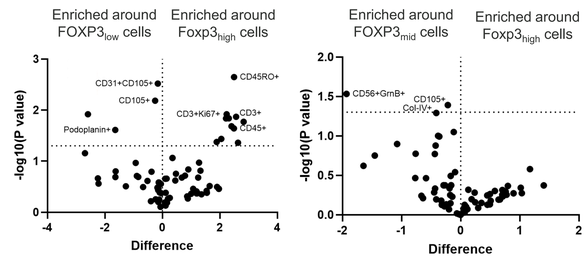
$FOXP3_{mid}$ niches are enriched CD56+GranzymeB+ cells and remodeling vasculature



$FOXP3_{high}$ niches are enriched in adaptive immune cells and proliferating T cells



FOXP3 expression levels map onto distinct spatial and phenotypic cellular neighborhoods



TGFβ3 transcript abundance decreases progressively from $FOXP3_{low}$ to $FOXP3_{high}$ Tregs

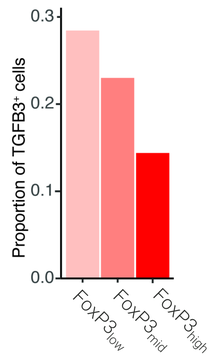


Fig. 5 $FOXP3_{low}$ Tregs show the highest proportion of TGFβ3+ cells, decreasing progressively through $FOXP3_{mid}$ to $FOXP3_{high}$.

TGFβ3 drives pro-inflammatory Th17 differentiation via IL-23/IL-6 signaling, linking $FOXP3_{low}$ Tregs to immune instability rather than canonical suppression.

References

1. Christians et. Al. Development of a novel tri-omic spatial biology assay that provides in-depth immune profiling of the tumor immune microenvironment. AACR (2025).
2. Li et. al. Spatial transcriptomics atlas of inflammatory bowel disease to guide implementation in research consortiums and clinical trials. Nat. Comms (*in press*)

Conclusion

- Quantitative FOXP3 reveals that $FOXP3_{low}$, $FOXP3_{mid}$, and $FOXP3_{high}$ Tregs occupy spatially and functionally distinct niches within the inflamed colon.
- $FOXP3_{low}$ and $FOXP3_{mid}$ Tregs, enriched in stromal-vascular, cytotoxic, and remodeling niches and biased toward TGFβ3, suggest a destabilized pro-inflammatory Treg state.
- Cross-platform spatial multi-omics uncovers context-dependent Treg heterogeneity invisible to either modality alone.

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