Single-Cell Spatial Analysis of Plasma Cell Organization in Colorectal Cancer Recurrence

Amruta Velamuri, Ellie Chan, Louis Vaickus, Joshua Levy, Emerging Diagnostic and Investigative Technologies, Department of Pathology, Dartmouth Hitchcock Medical Center

Dartmouth Health

ABSTRACT

- Single-cell spatial transcriptomic data from G4X colorectal cancer samples (n=127 sections, 78–97 patients) were analyzed to identify spatial features associated with recurrence
- A plasma-cell-rich niche and spatially clustered IgG plasma cells were significantly protective against recurrence (p=0.006)
- This work identifies plasma cell spatial organization - not abundance alone - as a candidate recurrence-protective feature in CRC

INTRODUCTION

Colorectal Cancer (CRC):

- 3rd most common cancer, 2nd leading cause of cancer-related deaths worldwide
- Recurs in up to 30% of Stage II patients even after complete tumor removal
- Current TNM staging cannot predict which patients' cancer will return

The problem with Recurrence

- Two patients with identical tumor stages can have completely different outcomes
- No reliable way to identify who is at risk for early intervention

Tumor Microenvironment (TME):

- The spatial organization of immune cells may predict recurrence beyond cell counts alone
- Prior work shows spatial context predicts recurrence better than biomarker count

Goal: Use single-cell spatial transcriptomics to identify spatial cellular niches associated with colorectal cancer recurrence

- **Single-Cell Spatial Transcriptomics (G4X):**
  - Measures gene expression of individual cells while preserving their spatial location in tissue
  - 365-gene targeted panel captures tumor, immune, and stromal cell types
  - Enables mapping of cellular niches and their spatial organization
  - Resolves plasma cells by immunoglobulin isotype (IgG, IgA, IgM)

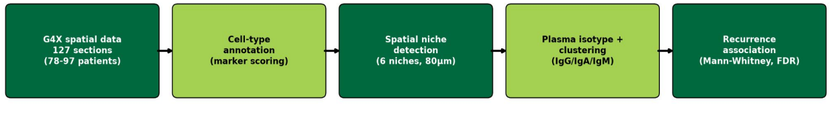


Figure 1: Analysis pipeline - from G4X spatial data to recurrence association.

METHODS

Data: 127 G4X single-cell spatial transcriptomic sections from the ColoCare CRC cohort (78–97 patients with matched recurrence outcomes); 365-gene targeted panel capturing tumor, immune, and stromal cell types

Preprocessing & Cell-Type Annotation:

- Cells filtered by minimum counts and genes; normalized and log-transformed
- Per-cell marker-gene scoring assigns each cell to a type (tumor epithelial, fibroblast, macrophage, T cell, plasma cell)

Niche Detection:

- Spatial neighbor graph built per section (80 µm radius)
- Neighborhood composition vectors assign each cell to one of six spatial niches, using discovery-cohort niche centers

Plasma Cell Isotype & Spatial Organization:

- Plasma cells classified as IgG, IgA, or IgM by argmax of IGHG1/IGHA1/IGHM
- Spatial clustering quantified as the fraction of a cell's neighbors sharing its isotype (tertiary-lymphoid-structure-like metric)
- IgG/IgA localization relative to tumor and T cells evaluated

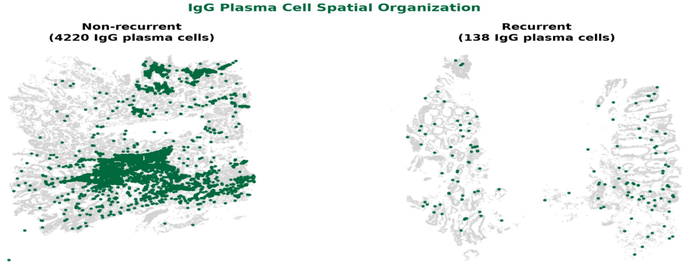
Recurrence Association:

- Per-patient niche and isotype features compared between recurrent and non-recurrent patients (Mann-Whitney U, Benjamini-Hochberg FDR correction)
- Random Forest with leave-one-out cross-validation and SHAP feature importance to assess recurrence prediction

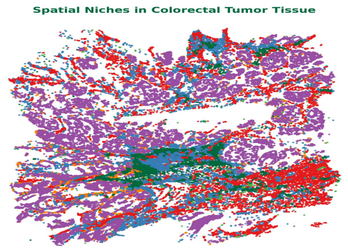
RESULTS

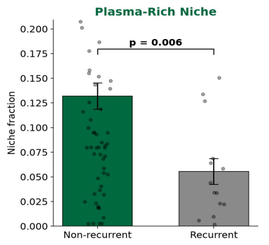
Key Finding: A plasma-rich niche is protective against recurrence

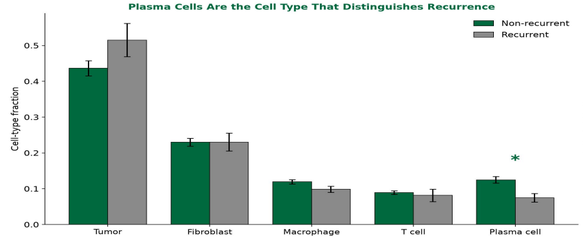
- Recurrent tumors had less than half the plasma-rich niche of non-recurrent tumors (p=0.006, FDR-corrected)
- Only niche to survive correction across all six niches
- Other niches null (tumor core p=0.11, myeloid p=0.42, T-cell p=0.43, CAF p=0.87)

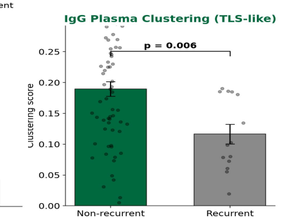


RESULTS









CONCLUSION

Limitations:

- Modest number of recurrence events (14–17), limiting power for subgroup analyses
- MSI status unavailable in the cohort metadata - a key potential confounder that could not be tested
- Single retrospective cohort; findings require external validation

Future Directions:

- Validate the plasma-rich niche and IgG-clustering findings in larger and cross-platform cohorts (e.g., Xenium), which would establish whether the signal is robust across spatial-profiling technologies
- Formally evaluate the IgG:IgA plasma cell ratio as a standalone, quantitative recurrence biomarker that could be computed from a simpler, pathologist-deployable assay
- Incorporate MSI status, tumor stage, and other clinical variables to determine whether plasma cell spatial organization predicts recurrence independently of established prognostic factors

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