Multi-omics spatial analysis of colon cancer tissue reveals emergence of an immunosuppressive tumor maintenance system

Gaurav Joshi[1], Nicholas Sciascia[1], Michael Yang[1], Brian Falkenstein[2], Raymond Yan[2], Shannon Quinn[2], Brenna Fearey[1], Junya Yoshioka[1], Akif Burak Tosun[2], S. Chakra Chennubhotla[2], Filippo Pullara[2], Fumiki Yanagawa[1]

Nikon Instruments, Inc., Boston, MA[1], PredxBio Inc, Pittsburgh, PA[2]

Abstract

Using a combination of modalities such as sequential multiplex immunofluorescence (mIF) and spatial transcriptomics (STx) on colon cancer sample we investigated spatial distribution, phenotype, function, and gene expression profile within the colon cancer and healthy tissue. Immunosuppressive cells, Treg and M2 macrophages were in the TME. Presence of other immune cells was not sufficient to prevent crypt hyperproliferation. While immunosuppressive phenotype was observed using antibodies, a strong presence of immunosuppressive genes was not found in transcriptomic study. Analysis of this multi-omics dataset showed hypoxia, a known factor to drive immunosuppression and COL6A2 gene expression to have strong association in driving tumor progression.

Multi-omics Spatial Imaging and Analysis

Lunaphore COMET

Visium CytAssist

Lunaphore HORIZON™
10x Loupe Browser
PredxBio SpaceIQ™

Data Analysis

Antibody Panel

Checkpoint	Structural	Stromal	T cells	Autophagy & Hypoxia
PD-1	E-Cadherin	α-SMA	CD3	CA-9
PD-L1	PanCK	Podoplanin	CD4	LC3B
IDO-1	**NK Cell**	**Neutrophil**	CD8	**Endothelial**
VISTA	CD56	CD66b	FOXP3	CD31
ICOS	**B Cell**	CD177	Treg	
CTLA-4	CD20		TCR delta	
TIM-3			Granzyme B	

5 um FFPE sections were prepared from stage IIA colon cancer sample of a 56-year-old individual exhibiting moderate to poorly differentiated adenocarcinoma of T3, N0 staging. The tumor was microsatellite stable (MSS) and mismatch repair proficient (pMMR). There was low tumor mutational burden.

SpaceIQ™, PredxBio's explainable AI-driven spatial multi-omics platform that provides a rigorous framework to interrogate the TME, is utilized to analyze data. By leveraging probabilistic modeling and unbiased cell typing, SpaceIQ™ uncovered transition cell states, cell–cell interactions, and mechanistic pathway networks within the tumor – yielding a deeper understanding of tumor heterogeneity and therapy resistance.

References

1. Li X, Li Z, Gu S, Zhao X.. A pan-cancer analysis of collagen VI family on prognosis, tumor microenvironment, and its potential therapeutic effect BMC Bioinformatics. 2022 Sep 27;23(1):390 PMID: 36167487

NIKON BIOIMAGING LAB

YOUR SPATIAL BIOLOGY CRO PARTNER

Contact: gaurav.joshi@nikon.com

DOWNLOAD POSTER

Results

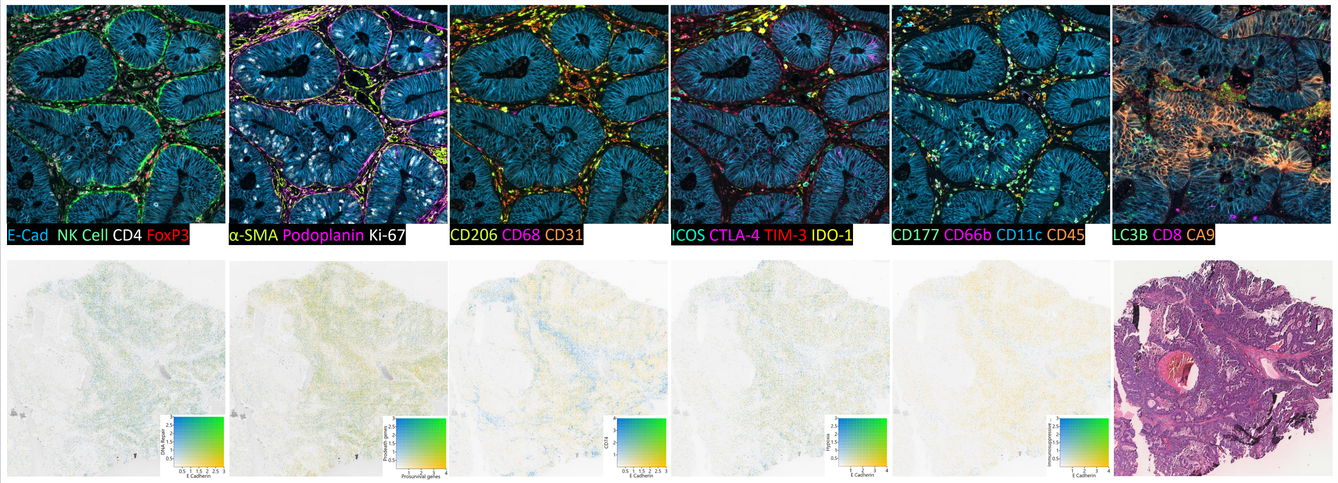


FIGURE 1 - Multiplex IF and Visium HD on adjacent sections from colon cancer sample show hyperproliferated crypts, diversity of TME, and gene expression results for various mediators of tumor progression: E-Cadherin as a marker of epithelial cells is labelled in Cyan highlighting the crypts. Various immune cells and stromal markers surround the crypt in TME. With an exception of CD177+ neutrophils, none of the immune markers used in this study invade the tumor. Stromal proteins such as α-SMA and Podoplanin surround the hyperproliferated crypt. For the Visium HD data, co-expression matrix of various genes in relation to E-Cadherin shows their distribution in TME.

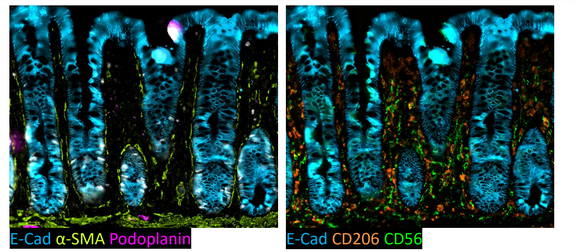
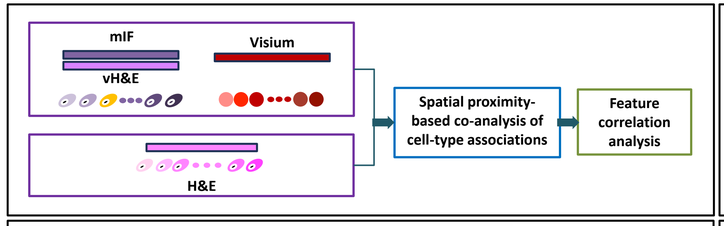
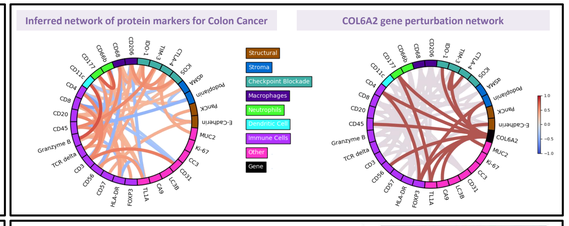
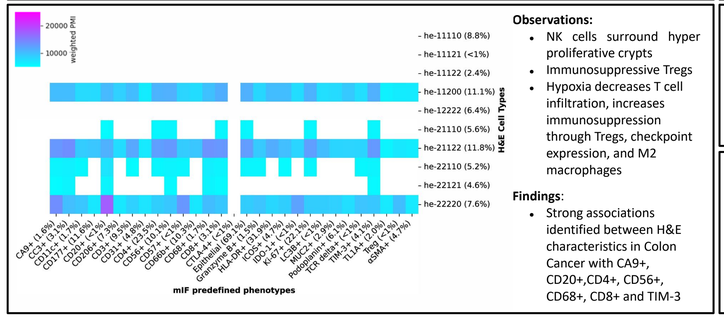


FIGURE 2 – Multiplex IF and Visium HD on adjacent sections from a healthy colon tissue show variation in localization and presence of proteins compared to diseased tissue: α-SMA+ cells are present around the crypt, at a lower density and further from cyrpt, while Podoplanin is absent. M2 macrophages and NK cells were present in lamina propria.

Multi-omics Spatial Analysis with PredxBio's SpaceIQ™ Platform







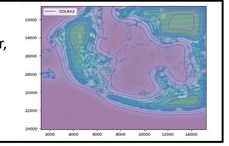
Observations:

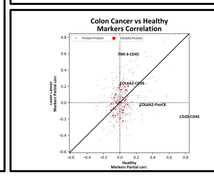
- NK cells surround hyper proliferative crypts
- Immunosuppressive Tregs
- Hypoxia decreases T cell infiltration, increases immunosuppression through Tregs, checkpoint expression, and M2 macrophages

Findings:

- Strong associations identified between H&E characteristics in Colon Cancer with CA9+, CD20+,CD4+, CD56+, CD68+, CD8+ and TIM-3

Spatial distribution of cells expressing COL6A2 were different between healthy and colon cancer, and they interacted differently with other cells. This spatial context is important to identify gene correlation instead of measuring absolute gene expression in a non-spatial context.





Observations:

- NK cells surrounding hyperproliferative crypts
- Immunosuppression of TME

Findings:

- Colon cancer : Increased TIM-3 checkpoint and NK cells-COL6A2 correlation
- Healthy colon : Increased B cells and epithelial-COL6A2 correlation

FIGURE 3 - SpaceIQ platform identifies COL6A2 gene and hypoxia as key mediators in disease progression: A pointwise mutual information (PMI) for probabilistic cell-cell association was derived analyzing various features obtained from H&E cell typing. Overlaying the PMI on mIF or Visium HD data allowed us to identify COL6A2 gene and CA9 protein involved in hypoxia as key mediators

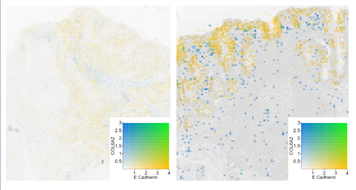


FIGURE 4 - Comparison of COL6A2 expression between diseased and healthy colon tissue: COL6A2 is highly expressed in the space around the crypts in TME occupied by stroma and immune cells. There is also some expression of COL6A2 in lamina propria and Peyer's patch

Conclusions

- Comparison between stage IIA, pMMR colon cancer and healthy colon tissue, show structural changes with immune cells and fibroblasts enriched TME. These give an insight in to tumor progression, it's potential to metastasize and help find ways to mitigate it.
- CAFs interact with immune cells to create an immunosuppressive environment promoting cancer growth. Drugs targeting CAFs to inhibit/reprogram them are an active area of research.
- SpaceIQ platform identified CA9, hypoxia marker and COL6A2 gene for having strong feature associations. COL6A9 has been shown to contribute towards tumor progression by vascular remodeling from ECM, promoting drug resistance[1].

PredxBio

Reimagining Cancer Care with AI-Driven Spatial Biomarkers

Contact: fillipo@predxbio.com

SpaceIQ™ platform