

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

### Abstract

Using a combination of modalities such as sequential multiplex immunofluorescence (mIF) and spatial transcriptomics (STx) we investigated spatial distribution, phenotype, function, and gene expression profile within the colon cancer and healthy colonx tissue. Immunosuppressive cells, Treg and M2 macrophages were in the TME. Presence of other immune cells was not sufficient to prevent crypt hyperproliferation. Immune cell infiltration into the crypt was prevented by a stromal cell barrier. While immunosuppressive phenotype was observed || B using antibodies, a strong presence of immunosuppressive genes was not found in transcriptomic study. The latter showed gene expression signatures related to processes of DNA damage, cell death, and hypoxia in epithelial cell regions.

## **Multi-omics Spatial Imaging and Analysis**

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

5 um FFPE sections were prepared from stage IIA colon cancer sample of a 56-yearold 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.





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### **Results and Conclusions**



FIGURE 1 – Colon cancer sample show differentially proliferated crypts, TME diversity, immune cell evasion and gene expression signatures related to various processes using multi-omics imaging and transcriptomics



FIGURE 2 – Healthy colon sample show organization of crypts, supporting stromal cells, and a diverse immune cell population in the Peyer's patch

- Hyperproliferated crypts in colon cancer: E-Cadherin as a marker of epithelial cells is labelled in cyan highlighting the crypts. Various immune cells and stromal markers surround the crvpt in TME. With an exception of CD177+ neutrophils, immune cells did not invade the crypt, nor kill the epithelial cells. Stromal proteins such as  $\alpha$ -SMA and Podoplanin surround the hyperproliferated crypt. expressing various checkpoint proteins were present in TME.
- B) Non-hyperproliferated crypts in colon cancer: Intensity of E-Cadherin on nonhyperproliferated crypt was less than that on the hyperproliferated region. NK cells were not present in this zone. The organization of the stromal proteins around the crypts include high density of Podoplanin expressing cells, and  $\alpha$ -SMA positive cells that do not surround Anti-inflammatory M2 along with expressing various checkpoint proteins were present in the TME.
- C) Spatial transcriptomics analysis: For the Visium HD data, co-expression matrix of various genes in relation to E-Cadherin show their distribution in TME. Clustering genes based on their biological relevance show prevalence of DNA repair pathways, pro-survival genes in epithelial cells, and tissue wide upregulation of hypoxia related genes.

- A) Organization of epithelial cells and various immune cells: E-Cadherin as a marker of epithelial cells is labelled in cyan highlighting the crypts. α-SMA surround the crypt providing a necessary structural support, with a noticeable absence of Podoplanin. The lamina propria consisted of M2 macrophages and NK cells. A diverse population of immune cells were found in the Peyer's patch, a type of secondary lymphoid organ.
- B) Spatial transcriptomics analysis: For the Visium HD data, co-expression matrix of various genes in relation to E-Cadherin show their distribution in the epithelial cell region or the Peyer's patch. CD74 expression was found comparable to that of the colon cancer sample in the Peyer's patch enriched in immune cell. CD74 is a favorable predictive marker for immunotherapy response in colon cancer. This data show that CD74 enriched cell population is also present in the healthy colon, and do not appear during cancer progression.