Interrogating microbiome-driven tertiary lymphoid structure formation in colorectal cancer

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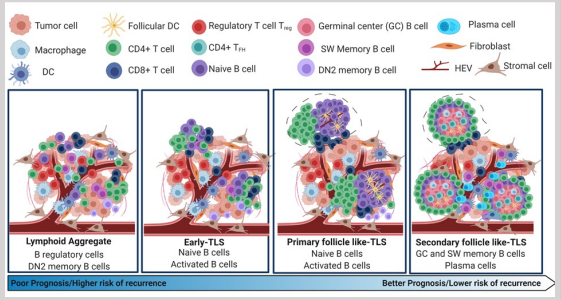
Introduction

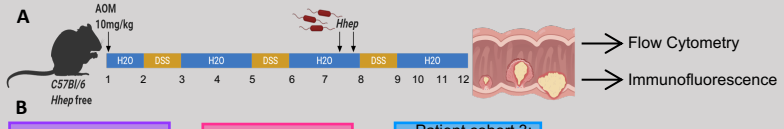
- Colorectal cancer (CRC) is one of the leading causes of cancer related death and end-stage disease is largely refractory to current treatments.
- As a barrier surface tumor, the tumor directly interacts with the organisms in the microbiome.
- Recent research suggests the microbiome may predict patient response to immunotherapy and contributes to either an immunosuppressive or immunocompetent tumor microenvironment (TME)[2,3,4].
- The presence of tertiary lymphoid structures (TLS), organized clusters of immune cells that allow for maximal T and B cell activation, have also been correlated with enhanced patient response to immunotherapy.
- We have shown in a mouse model of CRC that the modification of the microbiome with an immunogenic mucosal residing organism, *Helicobacter hepaticus (Hhep)*, leads to increased survival, reduction in tumor burden, increased immune infiltration in the TME and a significant increase in the formation of TLS within and around the tumor[1].



Hypothesis

By identifying specific organisms within the microbiome that activate effective anti-tumor immunity through maturation of tertiary lymphoid structures, we can identify potential targets for microbiome- or TLS-driven therapies to enhance patient responsiveness to immunotherapy.

Methods



B

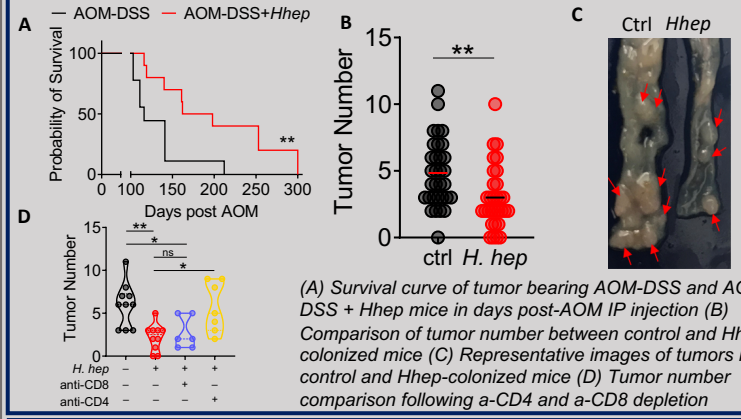
Patient cohort 1: 30 newly diagnosed rectal cancer patients (retrospective)

Patient cohort 2: Whole tissue from CRC tumors (prospective)

Patient cohort 3: Newly diagnosed rectal cancer patients (pre- and post-TNT)

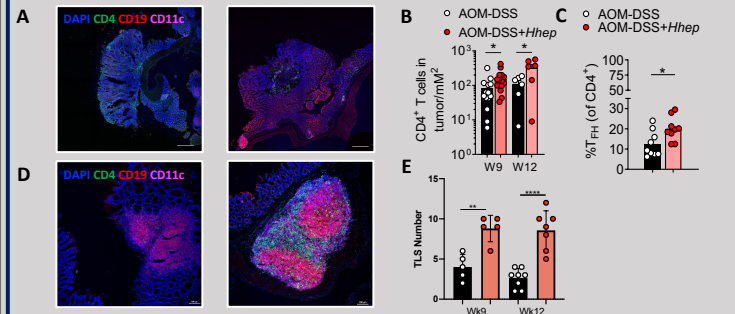
→ Flow Cytometry
Multi-spectral imaging
Microbiome analysis

Figure 1: Colonization with *Helicobacter hepaticus* provides a CD4⁺ T cell dependent survival advantages and reduces tumor burden



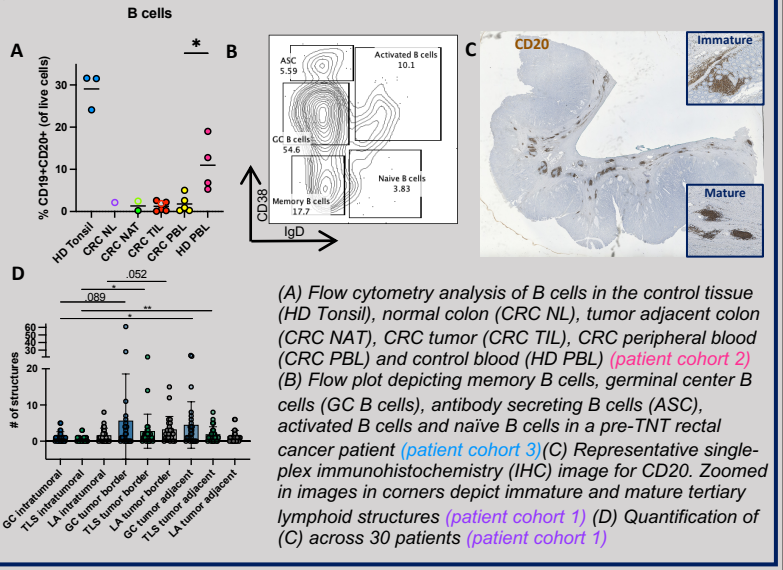
(A) Survival curve of tumor bearing AOM-DSS and AOM-DSS + Hhep mice in days post-AOM IP injection (B) Comparison of tumor number between control and Hhep-colonized mice (C) Representative images of tumors in control and Hhep-colonized mice (D) Tumor number comparison following a-CD4 and a-CD8 depletion

Figure 2: *Hhep*-colonization increases immune cell invasion and drives the maturation of T follicular helper (T_{FH}) cells that aid in the development of organized tertiary lymphoid structures



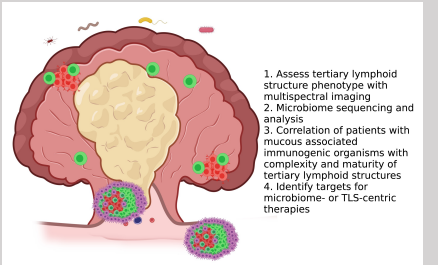
(A) Representative images of tumors from control and Hhep-colonized mice stained with DAPI (blue), CD4 (green), CD19 (red), CD11c (pink) (B) Quantification of CD4 infiltration in tumors (A) (C) Flow analysis depicting % T_{FH} of total CD4⁺ T cells in control vs Hhep-colonized mice (D) Representative images of TLS in control and Hhep-colonized mice stained with DAPI, CD4, CD19, and CD11c (E) Quantification of (D)

Figure 3: Rectal cancer patients have immature and mature tertiary lymphoid structures within the tumor microenvironment



(A) Flow cytometry analysis of B cells in the control tissue (HD Tonsil), normal colon (CRC NL), tumor adjacent colon (CRC NAT), CRC tumor (CRC TIL), CRC peripheral blood (CRC PBL) and control blood (HD PBL) (patient cohort 2) (B) Flow plot depicting memory B cells, germinal center B cells (GC B cells), antibody secreting B cells (ASC), activated B cells and naïve B cells in a pre-TNT rectal cancer patient (patient cohort 3)(C) Representative single-plex immunohistochemistry (IHC) image for CD20. Zoomed in images in corners depict immature and mature tertiary lymphoid structures (patient cohort 1) (D) Quantification of (C) across 30 patients (patient cohort 1)

Future Directions



1. Assess tertiary lymphoid structure phenotype with multispectral imaging
2. Microbiome sequencing and analysis
3. Correlation of patients with mucous associated immunogenic organisms with complexity and maturity of tertiary lymphoid structures
4. Identify targets for microbiome- or TLS-centric therapies

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