Interrogating microbiome-driven tertiary lymphoid structure formation in colorectal cancer

Hannah J. Bumgarner¹,³, Abigail E. Overacre-Delgoffe²,³, Sowmya Narayanan³, Ayana T. Ruffin⁴,⁵, Caleb Lampenfeld⁴, Timothy W. Hand²,³, Tulia C. Bruno²,⁴

¹ Medical Scientist Training Program, University of Pittsburgh School of Medicine, 2 Department of Immunology, University of Pittsburgh School of Medicine, 3 RK Mellon Institute for Pediatric Research, UPMC Children's Hospital of Pittsburgh, 4 Tumor Microenvironment Center, UPMC Hillman Cancer Center, 5 Program in Microbiology and Immunology, University of Pittsburgh School of Medicine

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)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 tumor⁴.

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

Structures, we can identify potential targets for microbiome formation in colorectal cancer

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

(A) Survival curve of tumor bearing AOM-DSS and AOM-DSS + Hhep mice in days post-ADM 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 depletions

Figure 2: Hhep-colonization increases immune cell invasion and drives the maturation of T follicular helper (Tfh) 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 % Tfh 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 TNT), CRC tumor (CRC TIL), CRC peripheral blood (HD PBL) and control blood (HD NBL) (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 naive 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)

Support: Research reported in this paper was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number T32GM008208. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

*Funding: Funding to support the work described in this manuscript was provided by 1) a Human Microbiome Program (HMP) grant from the National Institutes of Health under Award Number U54AI130024, and 2) a Medical Scientist Training Program (MSTP) grant from the National Institutes of Health under Award Number T32GM008208. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

**Cellular immunology post-mortem colorectal cancer (2018). Cell Metab. 29, 1–9. 10.1016/j.cmet.2018.07.015. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Future Directions

Flow cytometry analysis of B cells in the control tissue (HD Tonsil), normal colon (CRC NL), tumor adjacent colon (CRC TNT), CRC tumor (CRC TIL), CRC peripheral blood (HD PBL) and control blood (HD NBL) (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 naive 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)