Tissue-based characterization of T cell exhaustion in inflammatory bowel disease and colorectal cancer using multiplex IHC

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Abstract

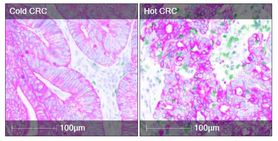
T cell exhaustion and the PD-L1/PD-1 checkpoint axis has been extensively characterized in peripheral blood mononuclear cells and in human tumor tissues. This has provided a better understanding of the role this pathway plays in tumor immunology and of its clinical utility in predicting responsiveness to checkpoint inhibitor therapies. T cell exhaustion is not only associated with tumor progression, but has recently been associated with better prognosis and milder course if disease for a number of autoimmune and autoinhibitory disorders. We set out to characterize and contrast the T cell exhaustion environment between colonic Crohn's disease (CD) and colorectal cancer (CRC). We applied the Ultivue UltiMapper multiplex fluorescence IHC platform to capture complex immune cell phenotypes and provide a more in depth characterization than traditional IHC.

Methods

Commercially sourced FFPE surgical resections for n=5 colonic CD patients (matched lesional (L) and non-lesional (NL) tissue) were compared to n=5 CRC tumor resections (3 Cold and 2 Hot tumors) using the Ultivue UltiMapper multiplex fluorescence immunohistochemistry platform. Two UltiMapper kits were used to evaluate the T cell environment in these tissues: UltiMapper I/O PD-L1 panel included the markers CD8, CD68, PD-L1, and pan-Cytokeratin/SOX10; UltiMapper I/O PD-1 panel included the markers CD3, CD45RO, PD-1, and pan-Cytokeratin/SOX10. All assays were stained on Leica BOND RX autostainers. Whole-slide images were acquired on a ZEISS Axio Scan.Z1 slide scanner. Image analysis was performed using Indica Labs HALO software. Statistical analyses used a non-parametric test with Kruskal-Wallis test to correct for multiple comparison (NL vs L, and Cold vs Hot).

Figure 1: Leica BOND RX autostainer, ZEISS Axio Scan.Z1 whole slide scanner, Indica Labs HALO software.

Controls



nuclei CD3 panCK/SOX10

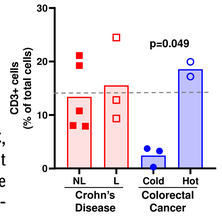
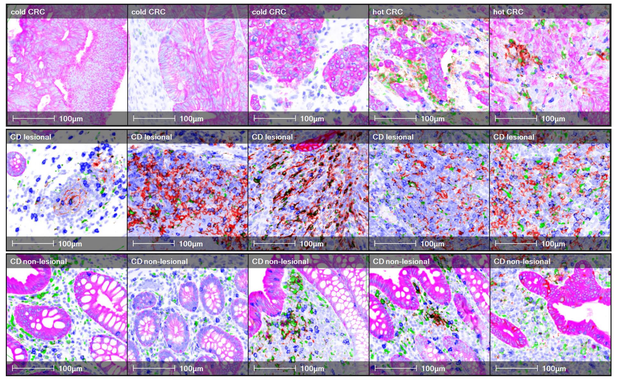


Figure 2: Representative images from a cold and hot CRC, visualizing only CD3 and panCK. The plot on the right shows the percentage of CD3+ T cells present in the tissues of cold vs. hot CRC. Hashed line represents non-IBD colon data, n=1.

Results

PD-L1 status of phagocytes and epithelial cells



nuclei CD8 CD68 PD-L1 panCK/SOX10

Figure 3: Image panels with representative images from cold and hot CRC, and lesional and non-lesional CD tissues, stained with the PD-L1 kit.

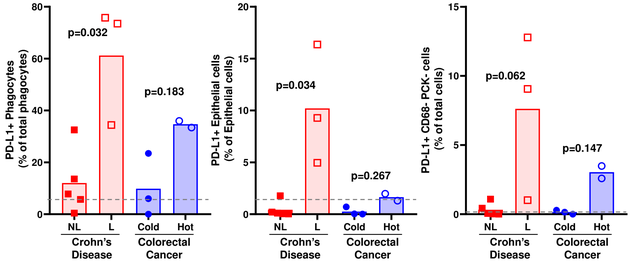


Figure 4: Cell phenotype quantification of CD and CRC tissues using the PD-L1 kit. There is an increase in the percentage of CD68+ cells that are PD-L1+ in lesional vs non-lesional CD, and hot vs cold CRC. PD-L1+ immunoreactivity in epithelial cells (panCK+) are similarly low in non-lesional CD and cold CRC and is increased in lesional CD. Hashed line represents non-IBD colon data, n=1.

CD8 cells and relationship to PD-L1 cells

Figure 5: Image panels above show representative nearest distance plot of CD8+ cells to PD-L1+ cells in cold and hot CRC, and lesional and non-lesional CD tissues stained with the PD-L1 kit.

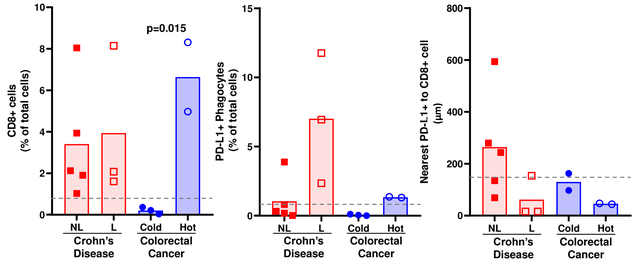
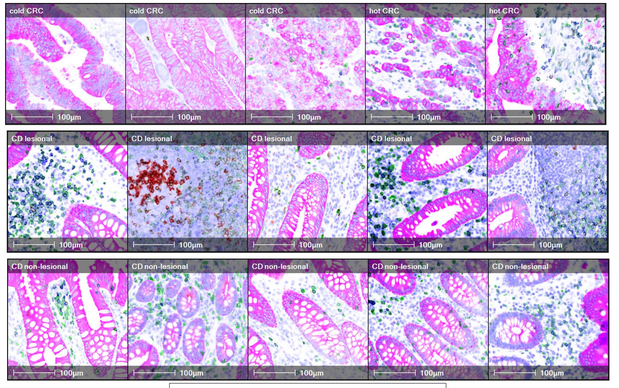


Figure 6: CD8 T cell quantification and spatial analysis in CD and CRC tissues with the PD-L1 kit. Lesional and non-lesional CD tissue as well as hot CRC show similar percentages of CD8+ cells; these are largely absent in cold tumors. CD8 T cells in cold tumors are also further, on average, from the nearest PD-L1+ cells (phagocyte or epithelial) than in hot tumors. In this respect cold tumors more resemble non-lesional CD and hot tumors more resemble lesional CD. Hashed line represents non-IBD colon data, n=1.

PD-1 status of CD3+ T cells



nuclei CD45RO CD3 PD-1 panCK/SOX10

Figure 7: Image panels above show representative images from cold and hot CRC, and lesional and non-lesional CD tissues stained with the PD-1 kit.

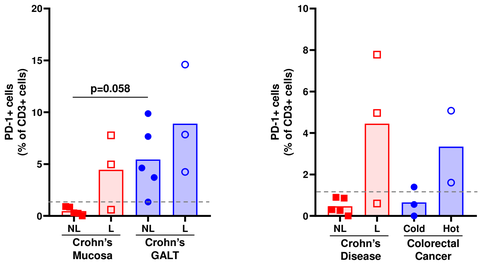


Figure 8: Cell characterization of CD and CRC tissues with the PD-1 kit. CD3 cells in the mucosa of non-lesional CD are less likely to be PD-1+ than lesional CD mucosa or gut associated lymphoid tissue (GALT). Despite cold CRC having many fewer CD3+ T cells, the PD-1 status of these CD3+ cells is similar to non-lesional CD. Hashed line represents non-IBD colon data, n=1.

Summary

- Ultivue UltiMapper PD-L1 and PD-1 kits are useful and effective tools to characterize tissue immune environment
- Hot CRC resembles lesional CD in terms of PD-L1 and PD-1 expression profiles
- PD-L1 and PD-1 status of cells in cold CRC resembles non-lesional CD
- Limitations of the study include the small n of samples; more samples are planned
- Limitations also include edge effect associated with spatial analysis