T cell response profiling in colorectal carcinoma patients reveals an enrichment in responses to specific tumor-associated antigens

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Introduction
Colorectal cancer (CRC) is one of the most common cancers worldwide and the second leading cause of cancer-related death in Western countries¹. While survival rates even for metastatic disease have greatly improved due to novel drugs and targeted therapies, tumor stage at time of diagnosis is still the most important predictor of treatment outcome. Several non-invasive diagnostic tests are in development for CRC focusing on detection of soluble macromolecules or circulating tumor cells. Here, we explore the utility of tumor-associated antigen (TAA)-specific T cell response profiling for vaccine candidate identification and as an alternative for early non-invasive detection of CRC.

Several studies have established the presence of intratumoral T cell infiltrates as a prognostic factor for CRC³, and TAA-specific responses in peripheral blood can be readily detected⁴. Here, we profile T cell recall responses to a set of TAAs in subjects with various stages of CRC and pre-malignant lesions in an HLA-independent manner. We utilize the ATLAS technology, with the proven ability to identify both CD4⁺ and CD8⁺ recall responses to a variety of antigen types, including neocarignins.

Methods: The ATLAS™ Platform

Figure 1: The ATLAS screening workflow. Whole blood was collected and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. CD4⁺ and CD8⁺ T cells were sorted and non-specifically expanded. CD4⁺ monocytes were differentiated into monocyte-derived dendritic cells (MDDCs). Full-length open reading frames of 25 putative antigens previously implicated in CRC were cloned and expressed in E. coli. Library clones were screened in duplicate using 500,000 E. coli, 1,000 - 5,000 MDDCs and 80,000 T cells per well. 20 replicates of E. coli expressing Neon Green (NG) were included as negative controls. Assay supernatants were harvested after 16-24 hours and IFN-γ and TNF-α concentrations were quantified using a custom Luminex platform.

Figure 2: Lysteriolysin O (LLO) facilitates MHC class I presentation by monocyte-derived dendritic cells (MDDCs). Co-delivery of a putative antigen and LLO by E. coli leads to pore formation in the phagosomal membrane upon acidification. Antigen is released into the cytosol, enzymatically processed and loaded on the surface's MHC class I molecule for presentation by autologous CD8⁺ T cells. Figure 3: The data analysis workflow. Individual steps of the data analysis process are listed.

Figure 4: Response profiles to 25 CRC-associated TAAs across CRC patients. CD4⁺ and CD8⁺ T cells from CRC patients across all stages of disease were profiled for responses to 25 TAAs, using IFN-γ and IFN-γ secretion as an indicator for a recall response to a putative antigen. Distributions of normalized cytokine concentrations released in response to each antigen are shown, each row represents one antigen. Dashed vertical lines indicate 2 MADs from median cytokine release in response to the NG negative control antigen. Positive values, indicated by a shift toward the right side of the plot, indicate stimulatory recall responses. Negative values, indicated by a shift toward the left side of the plot, indicate inhibitory responses.

Figure 5: High frequency of T cell responses to two novel TAAs. Response rates in individuals with CRC to two ATLAS-identified TAAs in comparison to three TAAs that are or were in clinical development as a therapeutic vaccine. Stimulatory (top panel) and inhibitory (bottom panel) T cell recall responses are shown.

Figure 6: T cell responses to selected TAAs in CRC patients with early or late stage disease. Stimulatory response rates in 4 selected TAAs are shown for both CD4⁺ and CD8⁺ T cell subsets and IFN-γ and IFN-γ release. Patients are grouped by stage of disease with early stage representing stages I and II, i.e. inoperable disease, and late stage representing stages III and IV, i.e. with metastasis to lymph nodes or distant sites. There was no significant difference between response rates in early and late disease for either stimulatory responses (shown) or inhibitory responses (not shown). NR, non-responders.

Figure 7: T cell responses to selected TAAs in healthy individuals and donors with various disease states. Normalized cytokine concentrations released in response to a selected TAA in the three cohorts are shown for CD4⁺ and CD8⁺ T cell subsets and for IFN-γ and IFN-γ release. Each data point represents one individual. IFN-γ release in different cohorts is compared using a Wilcoxon rank sum test. Asterisks indicate statistical significance in comparison to cytokine release in healthy donors unless otherwise indicated: * p < 0.05; ** p < 0.01; *** p < 0.001. Significant differences based on IFN-γ were detected across the same groups (not shown).

Acknowledgements and References

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¹ Do Divitiis et al., 2014; ² Reukerholter et al., 2015; ³ Nagenes et al., 2003; ⁴ Nagenes et al., 2005;
⁵ Hu et al., 2004; ⁶ Speetjens et al., 2011.

Conclusions

• HLA-independent T cell response profiling to a selection of TAAs implicated in CRC was performed in healthy donors and in individuals with CRC and adenomatous polyps.
• In CRC patients, the breadth of recall responses to these TAAs varied, but there was a strong enrichment of CD4⁺ and CD8⁺ T cell responses to a subset of TAAs, which was absent in healthy individuals.
• Stage of cancer did not impact the T cell response signature.
• Importantly, T cell responses to a subset of TAAs in individuals with pre-malignent adenomatous polyps were similar to those in CRC patients and clearly distinguishable from the rare responses in healthy individuals. This pattern was not observed for responses to TAAs currently or previously investigated as therapeutic vaccines.
• We are currently exploring whether age is a cofactor in this analysis.
• The emergence of a specific T cell response profile to a subset of TAAs opens the possibility for the development of a non-invasive blood-based assay to support early detection and diagnosis of CRC.
• Additionally, these TAAs may represent candidates for the development of an immunotherapeutic to complement personalized cancer vaccine approaches.