High density of CD68+HLA-DR+ macrophages in the stroma of primary melanoma correlates with an unfavorable immune microenvironment as assessed by Digital Spatial Profiling

Emanuella M. Rizk1, Andrew Chen1, Andrew M. Silverman2, Douglas K. Marks3, Raoul Rabadan1, Kit Fuhrman4, Alison VanSchoiack4, Yan Liang4, Joseph Beechem4, Yvonne M. Saenger2, Robyn D. Gartrell-Corrado2

1 Columbia University Irving Medical Center, New York, NY; 2 Columbia University Irving Medical Center/New York Presbyterian, New York, NY; 3 New York University Winthrop Hospital, New York, NY; 4 Nanoscint Technologies, Seattle, WA

Abstract 2978

Background

- Melanoma is the sixth most common form of skin cancer in the United States, and patients diagnosed with stage II-III melanoma have an approximate 35% risk of disease specific mortality.
- Prognostic tools to identify which patients with stage II-III melanoma are at greatest risk of recurrence will help stratify patients for adjuvant immunotherapy treatment.
- Previously, we created a database of 104 patients at Columbia University Irving Medical Center (CUIMC) with stage II or III melanoma for whom primary melanoma tissue specimens were available (Mantra cohort).
- Using quantitative multiplexed immunofluorescence (qmiF), we stained slides from the patients in the Mantra cohort with a panel of immune biomarkers (CD3, CD8, CD68, Ki67, SOX10 and HLA-DR) and analyzed the tumor stroma.
- Close proximity of HLA-DR+ macrophages to cytotoxic T lymphocytes (CTLs) is a marker of poor prognosis in patients with stage II-III melanoma (n=61, AUC=0.682, P=0.011; Figure 1A).
- A high CTL/macrophage ratio is a marker of good prognosis in patients with stage II-III melanoma (n=43, AUC=0.724, P=0.026; Figure 1B).
- In this work we further characterize the tumor immune microenvironment of patients with stage II-III melanoma using qmiF and Digital Spatial Profiling (DSP), specifically focusing on HLA-DR+macrophages in the tumor stroma (Figure 1C).

qmiF methods

Patient cohort:
A sub-cohort of 13 patients from the Mantra cohort was selected, enriching for patients with high density of CD68+HLA-DR+ macrophages. qmiF staining was performed on all 13 patients in this cohort, and DSP was performed on 8 patients from this cohort.

qmiF staining and image analysis:
5 micron full-section slides were stained using Opal qmiF for DAPI, CD68 (macrophages), HLA-DR (antigen presentation and activation), CSF1R (inflammation), CD163 (M2 macrophages), CD3 (T lymphocytes), and MHC-I (antigen presentation). H&E slides were reviewed by a dermatopathologist to determine representative areas for multispectral image (MSI) capture at 20x magnification using Mantra. MSI's were analyzed using inForm software and data was processed in Rstudio. Statistical analyses were then completed on GraphPad Prism, R Version 3.3.1, and Python 3.5.

DSP Methods

DSP cohort:
8 patients from the sub-cohort were selected for further analysis based on HLA-DR-macrophage phenotype and CD8 expression as determined by previous qmiF. 4 patients had a high density of HLA-DR+macrophages and close proximity of HLA-DR+ macrophages to CTLs (poor prognosis). The remaining 4 patients had a high CTL/macrophage ratio and low HLA-DR+ macrophage density, as determined by qmiF (good prognosis).

Using DSP to evaluate patients with high and low HLA-DR+ density:
Using DSP, we find that the ROIs from patients with high stromal HLA-DR+ macrophage density have lower overall immune infiltration, as assessed by quantitation of CD45 per ROI (p<0.0001).

Discussion

In this work we build on previous findings by examining the phenotypic differences between HLA-DR+ and HLA-DR- macrophages. Using qmiF, we find that HLA-DR+ macrophages in the tumor stroma express higher levels of CSF1R and CD33 than HLA-DR- macrophages. By combining these markers with CD163 we find greater overlap with HLA-DR+ macrophages, thus suggesting that a subset of these macrophages are M2 and likely immunosuppressive. Further, using DSP we compare overall protein expression in regions of interest in patient samples, and find that there is less immune infiltration and fewer T lymphocytes in patients with high levels of HLA-DR+ macrophages. Instead, patients with high levels of HLA-DR+macrophages express more immune checkpoint molecules per cell.

Conclusion

In this work we build on previous findings by examining the phenotypic differences between HLA-DR+ and HLA-DR- macrophages. Using qmiF, we find that HLA-DR+ macrophages in the tumor stroma express higher levels of CSF1R and CD33 than HLA-DR- macrophages. By combining these markers with CD163 we find greater overlap with HLA-DR+ macrophages, thus suggesting that a subset of these macrophages are M2 and likely immunosuppressive. Further, using DSP we compare overall protein expression in regions of interest in patient samples, and find that there is less immune infiltration and fewer T lymphocytes in patients with high levels of HLA-DR+ macrophages. Instead, patients with high levels of HLA-DR+macrophages express more immune checkpoint molecules per cell.

Acknowledgements

The study was funded by nanoString’s DSP InstaGene, the American Association for Cancer Research (AACR), and Columbia University Irving Medical Center (CUIMC). Robyn Gartrell-Corrado was funded by CUIMC K12 Scholar (K27R2031741) and Swim Across America.

References


Corresponding author: Robyn Gartrell, rgp2170@columbia.edu