Spatially Resolved Tumor-cell MHC Class II Shapes Adaptive Immunity and Therapeutic Response in Triple-negative Breast Cancer

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Abstract

Background: MHC class II molecules are normally restricted to professional antigen-presenting cells. Prior TNBC studies have focused on immune-compartment MHC-II. Using spatial transcriptomics, we recently identified aberrant tumor-cell MHC-II expression. Here, we characterized the immune architecture surrounding HLA-DRA-expressing tumor cells.

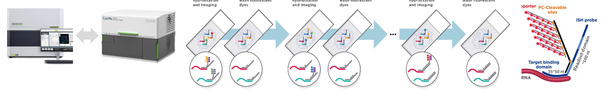
Methods: High-plex single-cell spatial transcriptomics (CosMx SMI) was performed on treatment-naïve TNBC (Mayo TMA, n=65) and two neoadjuvant pembrolizumab cohorts (Mayo n=8; Emory n=4). Spatial neighborhoods were mapped relative to HLA-DRA-high tumor cells. HLA-DRA-Top and –Bottom quartile were classified by RNA expression above or below 25th percentile. HLA-DRA-Top and –Bottom quartile were classified by RNA expression above or below 25th percentile. Differential expression and adaptive immune gene-set scores were evaluated. Clinical relevance was assessed using FinXX (n=114), I-SPY2 (n=364), and Caris CODEai (n=3,662).

Results: HLA-DRA-high tumor regions showed significant enrichment of adaptive immune subsets within ≤50 µm, including B cells, CD8 T cells, NK cells, macrophages, and plasmacytoid dendritic cells. In pembrolizumab-treated cohorts, responders exhibited higher proportions of HLA-DRA-high tumor cells and greater numbers of B cells, plasmablasts, CD4 and CD8 T cells, and macrophages near tumor cells. Responders' tumors showed upregulation of antigen-presentation machinery, B-cell/plasma-cell programs, CXCL13, and NOTCH3/DLL1. Across I-SPY2 and FinXX, higher HLA-DRA correlated with pathologic complete response and improved survival. In CODEai TNBC, high HLA-DRA expression correlated with higher CXCL13 (median TPM 7.5 vs. 1.1, q<0.05) and longer overall survival (24.2 vs. 18.5 months, HR 0.77, 95% CI 0.71-0.83, p<0.0001). This association was TNBC-specific. High CXCL13 also predicted improved survival (26.6 vs. 16.5 months, HR 0.64, 95% CI 0.59-0.69, p<0.0001).

Conclusions: Tumor-cell HLA-DRA expression defines a highly organized adaptive immune niche enriched with B cells and activated T-cell populations in close proximity. Adaptive immune programs—including enhanced antigen presentation and CXCL13-mediated B-cell/plasma-cell pathways—consistently associate with improved outcomes across TNBC cohorts treated with chemotherapy and immune checkpoint blockade. Aberrant tumor-cell MHC-II expression may contribute to antitumor immunity and warrants further investigation as a potential therapeutic target.

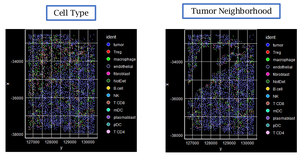
Methods

NanoString CosMx™ SMI for Single-Cell Imaging
CoxMx SMI is the fist high-plex *in situ* analysis platform that provide spatial multiomics using formalin-fixed paraffin-embedded (FFPE) and fresh frozen tissue samples at single-cell resolution Currently, this platform can evaluate up to 1,000 RNA and 64 proteins.

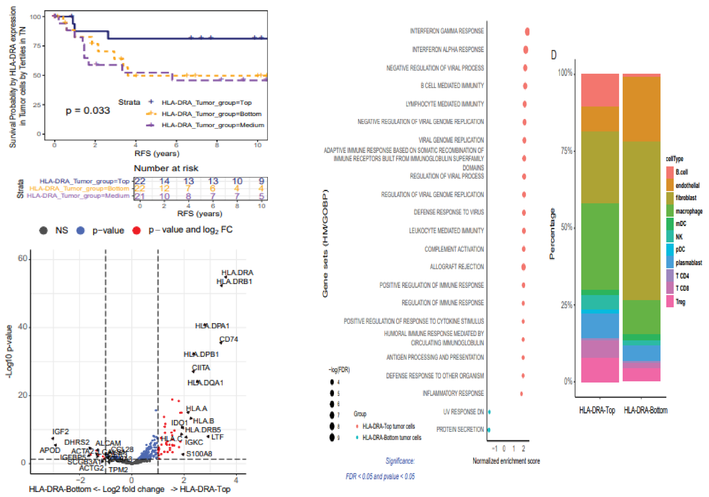


Comprehensive cell type and spatial neighborhood profile of TNBC TMA samples

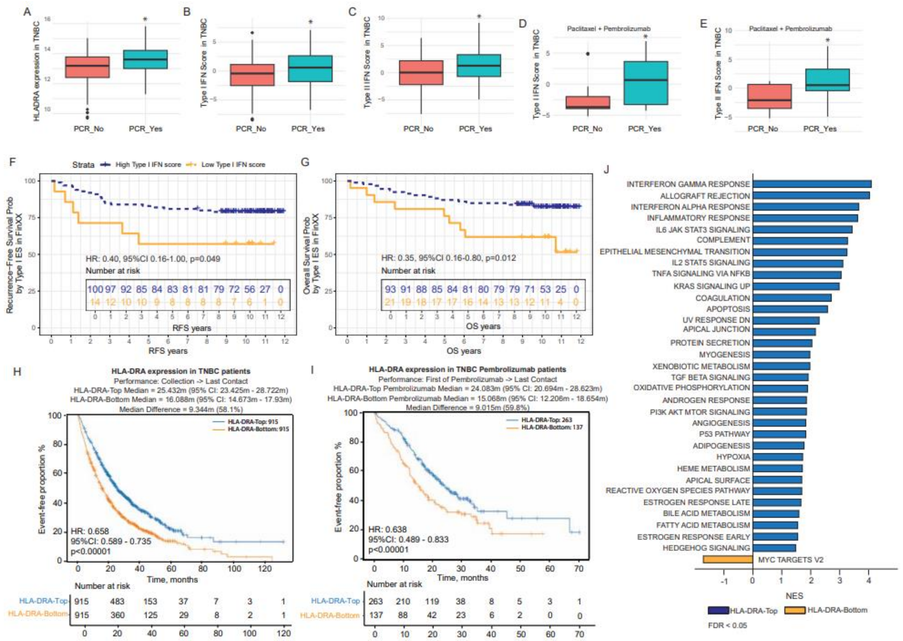
- Semi-supervised cell typing identified 12 cell types
- Two discrete cellular environments (Tumor Neighborhood and Stroma Neighborhood) within the TNBC samples.



Characteristics of Tumor Cells within the Tumor Neighborhood (TN) in the TNBC TMA Cohort



Clinical Relevance of HLA-DRA Expression and Interferon Signaling Across the I-SPY2, FinXX, and Real-world Cohorts



Conclusions

- **Novel TNBC-Specific Spatial Biomarker:** Tumor-cell HLA-DRA expression serves as a highly specific spatial marker for an active, interferon-inflamed microenvironment in Triple-Negative Breast Cancer (TNBC), and its prognostic value is independent of general stromal immune cell density
- **Drives a Localized Immune Circuit:** HLA-DRA-enriched tumor cells anchor a localized immune response driven by tumor-intrinsic cGAS-STING activation and IFNα production, which promotes distance-dependent immune activation and checkpoint (PD-L1) engagement in neighboring immune cells
- **Predicts Clinical Benefit for Immunotherapy:** High tumor-cell HLA-DRA expression is strongly associated with improved survival (RFS, EFS) and higher pathological complete response (pCR) rates to pembrolizumab-based therapies, offering a valuable tool to improve patient stratification for immune checkpoint inhibitors

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