Multiplexed ion beam imaging identifies B-cell enrichment in the RHAMM-high invasive niche of breast cancer

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Background

- Receptor for hyaluronan (HA) mediated motility (RHAMM, gene name *HMMR*) has been shown to work cooperatively with CD44 to mediate tumor progression [1]. However, its specific roles in breast cancer are still unclear.
- Our previous studies have shown tumor cell RHAMM deletion significantly inhibits cancer progression in xenograft models and that RHAMM is heterogeneously expressed within human breast tumors [2].
- We found that Type II Interferon signaling and MHC Class I & Class II Antigen Presentation pathways are co-enriched in the RHAMM high invasive niche. This indicates that RHAMM might be involved in regulating immune responses.

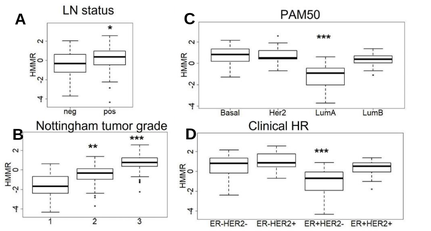

Figure 1. RHAMM expression is ssociated with aggressive breast ancer features. Gene expression was nalyzed in the UMN breast cancer patient ohort by Nanostring nCounter technology 2]. Levels of *HMMR* mRNA were ignificantly increased in association with: (**A**) lymph node positive status, (**B**) ncreased Nottingham tumor grade, (**C**) asal, HER2-enriched, and Luminal B ubtypes by PAM50, and (**D**) triple egative and HER2+ disease by clinical ormone receptor expression. *: p<0.05, **: p<0.01, ***: p<0.005.

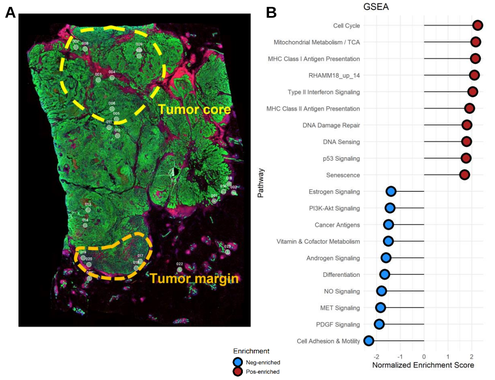

Figure 2. Spatial transcriptomic analysis of the RHAMM high niche in Triple Negative Breast Cancer(TNBC). (**A**) Low power image of a tumor stained for pan-cytokeratin (green), CD45 (red), and dsDNA (blue). Nanostring digital spatial profiling (DSP) was performed on regions of interest from the RHAMM-high tumor margin and RHAMM-low tumor core. (**B**) Enriched gene sets identified by comparing the RHAMM-high tumor margin vs. RHAMM-low regions identifies enriched interferon signaling and antigen presentation pathways.

Hypothesis

We hypothesize that upregulation of RHAMM expression is associated with an anti-tumor immune cell infiltration in breast cancer invasion.

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Study design and methods

FFPE tissues from 5 breast cancers were analyzed by multiple ion beam imaging (MIBI). Sections are stained with 20 antibodies simultaneously, including biomarkers to define immune cell phenotypes. Fields of views (FOVs) were selected from RHAMM high regions and RHAMM low regions on serial sections. Novel machine-learning-based algorithms were applied to segment the images into spatially resolved single-cell data and to classify immune cell populations in RHAMM-high and RHAMM-low regions.

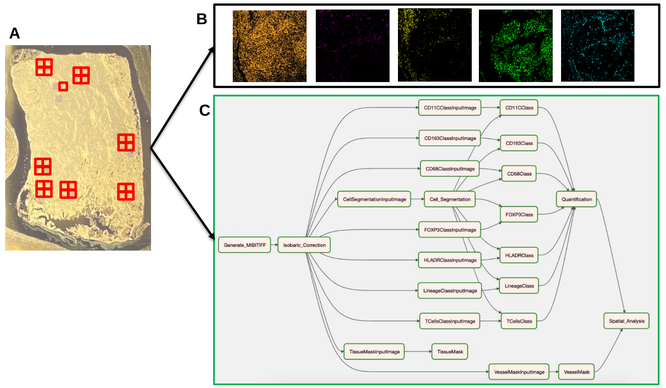

Figure 3. MIBI workflow. The stained slide (**A**) was scanned by the MIBIscope system (red squares label FOVs), visualized by MIBItracker (**B**), and segmented via the machine-learning-based algorithm (**C**). The widths of images in panel B are 800 µm.

Different immune infiltration patterns in tumor core and tumor margin

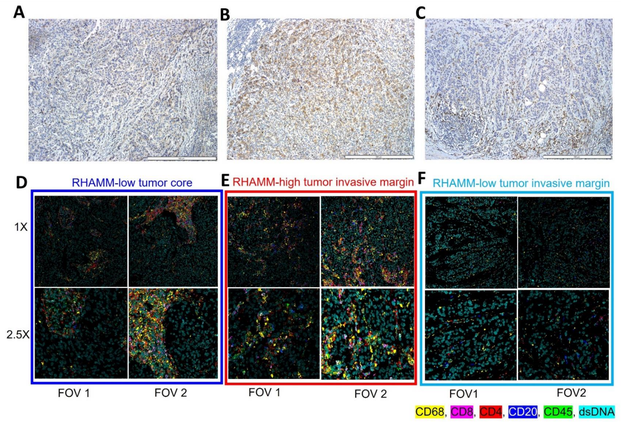

Figure 4. Comparison of RHAMM and Immune Marker Expression. Representative images of RHAMM IHC from the RHAMM-low tumor core **(A),** RHAMM-high tumor invasive margin **(B),** and RHAMM-low invasive margin **(C)**. The scale bar is 500 um. **D-F**: Corresponding MIBI images from these regions; relative zoom level from the MIBItracker user interface is indicated, and the 1X FOV is 800 um wide.

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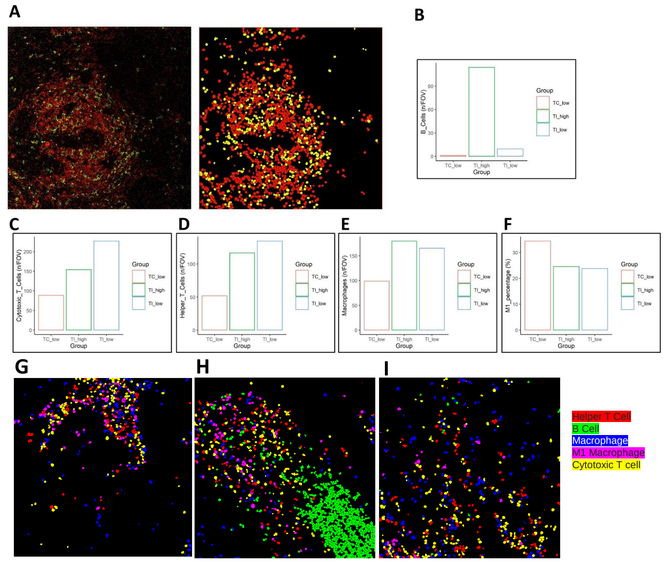

Figure 5. Immunophenotyping the breast tumor microenvironment. A: MIBI raw staining (left panel, red for CD8, yellow for CD4) and its corresponding digital segmentation (right panel, red for CD8+ T cells, yellow for CD4+ T cells). **B-F:** Quantification of B cells, cytotoxic T cells, helper T cells, macrophages, and % M1 (of total macrophages) of tumor core RHAMM-low (TC_low), invasive margin RHAMM-high (TI_high), and invasive margin RHAMM-low (TI_low) FOVs. **G-I**: Representative immune cell overlay plots of TC_low- TI_high, and TI_low FOVs, respectively.

Conclusions & Future Directions

Our data highlight potentially novel interactions between RHAMM expression/function and B-cell infiltrates in TNBC. Given the dynamic roles of B-cells in cancer immunology [3], this suggests new possible avenues for immune modulation to inhibit RHAMM-supported breast cancer progression. On-going work is confirming this linkage in additional TNBC and expanding the analysis to other molecular subtypes of breast cancer.

Acknowledgements & References

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1. Hamilton, S. R., Fard, S. F., Paiwand, F. F., Tolg, C., Veiseh, M., Wang, C., ... & Turley, E. A. The hyaluronan receptors CD44 and Rhamm (CD168) form complexes with ERK1, 2 that sustain high basal motility in breast cancer cells. J Biol Chem. 2007;282(22): 16667-16680.
2. Tarullo, S. E., He, Y., Daughters, C., Knutson, T. P., Henzler, C., Price, M., ... & Nelson, A. C. Receptor for Hyaluronan-Mediated Motility (RHAMM) defines an invasive niche associated with tumor progression and predicts poor outcomes in breast cancer patients. bioRxiv. 2022.
3. Yuen, G. J., Demissie, E., & Pillai, S. B lymphocytes and cancer: a love–hate relationship. Trends in cancer. 2016; 2(12): 747-757.