Targeting the Tumor Secretome to Enhance Immune Responses in Breast Cancer Liver Metastasis

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Introduction/Rationale

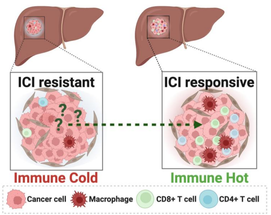
- More than 50% of metastatic breast cancers spread to the liver, a site that responds poorly to chemo and immunotherapy
- Liver metastases are considered immunologically cold, and this is thought to drive resistance; however, few studies investigate heterogeneity in the liver metastatic microenvironment
- We will overcome this knowledge gap by revealing mechanisms that drive an immune cold state at this metastatic site

Hypothesis

We hypothesize that comparison of immune hot and cold breast cancer liver metastases (BCLM) will reveal factors produced and secreted by the tumor to support an immune cold phenotype.

Proposed Project Model

Molecular vulnerabilities of immune suppression in BCLM



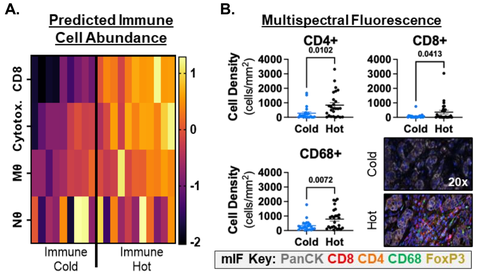
Project Model. We revealed that breast cancer liver metastases lie across a spectrum of immune infiltration with some being immune cold and others immune hot. This project compares differentially expressed factors between these immune phenotypes. We anticipate this approach will reveal additional targetable secreted factors that are molecular vulnerabilities of immune cold tumors.

Methods

- Human breast cancer liver metastases (BCLM) were profiled using NanoString nCounter and multispectral fluorescence imaging.
- Expression of the top differentially expressed gene, anterior gradient-2 (*AGR2*), was observed in additional BCLM, metastases from other sites, and BCLM patient derived organoids (PDOs).
- The impact of AGR2 on immune cells was assessed in publicly available BCLM scRNAseq (GSE249361) and by co-culturing T cells and spheroids.

1. Immune cold BCLM have elevated AGR2.

BCLM have heterogenous immune abundance



AGR2 is enriched in immune cold tumors

C. Genes Up in Cold BCLM

Gene	Log_2 Fold Change	-Log10 p-value
AGR2	6.40	1.85
APOD	3.43	1.89
CDK1	2.99	2.04
SIX1	2.71	1.99

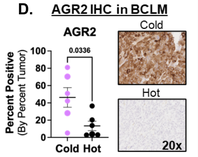
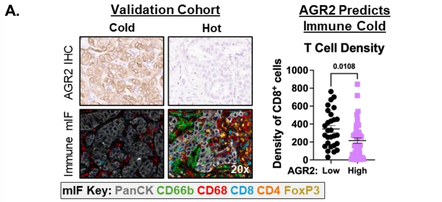


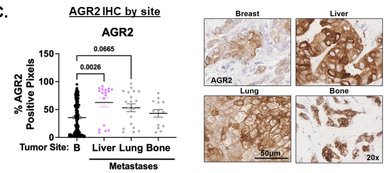
Figure 1. A-D. NanoString nCounter analysis and mIF were conducted on 20 BCLM (MΘ = macrophage, NΘ = neutrophil). Rosalind software revealed differential immune cell abundance (A). mIF confirmed immune cold and hot phenotypes (B). We assessed genes enriched in cold metastases (C) and confirmed AGR2 protein levels (D). Stats: unpaired Welch's t-test (B&D).

2. AGR2 is enriched in patient derived BCLM models and at additional metastatic sites.

AGR2 predicts T cell abundance in another cohort



AGR2 is upregulated at other metastatic sites



AGR2 in human BCLM models

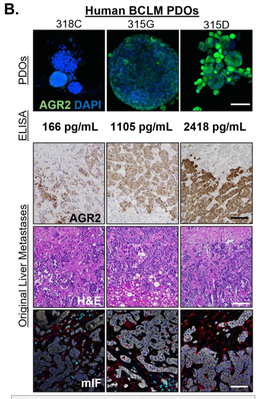
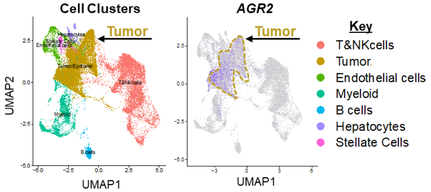
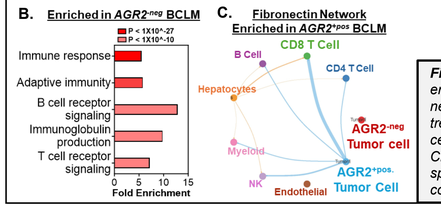


Figure 2. A. IHC for AGR2 and mIF for immune cell markers conducted on BCLM (N=19). We observed CD8⁺ cell density after stratifying tumors into AGR2-low (<10%+) and AGR2-high (>40%+) groups. B. AGR2 levels in three human BCLM PDOs via IF and in matching conditioned medium via ELISA (top). Matching FFPE samples assessed via IHC for AGR2, H&E, and mIF (bottom). 20x, all scale bars = 100 microns C. AGR2 IHC in a TMA from the CHTN. Stats: unpaired Welch's t-test (A) or ANOVA with Tukey's multiple comparison test (C).

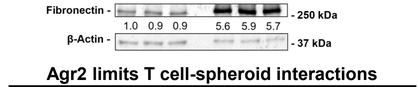
3. AGR2 impacts T cell infiltration and activation.

AGR2⁺ BCLM have altered T cell interactions





Agr2 enhances fibronectin production



Agr2 limits T cell-spheroid interactions

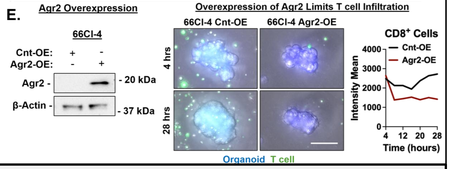


Figure 3. A. AGR2 levels in scRNAseq from six BCLM. B. Pathways enriched in AGR2-neg BCLM cells. C. CellChat was used to reveal signaling networks between AGR2-neg tumor and immune cells. D. 66Cl-4 cells were treated with 5 ug/mL rAgr2 for 72 hours. E. Agr2 overexpression in 66Cl-4 cells. Cells were stained with CellTrace Violet and grown as spheroids. CFSE dyed T cells were activated and added to organoids. Infiltration into spheroids was monitored. Shown is a representative organoid for each condition with the mean intensity of CFSE. Scale bar = 50 microns

Conclusions and Future Directions

- Comparison of immune hot and cold BCLM revealed *AGR2* as enriched in cold BCLM.
- Further assessment showed that AGR2 is maintained in BCLM PDOs and is elevated at other metastasis sites such as the lung.
- Preliminary mechanistic studies suggest that AGR2 may alter T cell infiltration and activation by modulating fibronectin production by tumor cells.
- **Ongoing and future work:** We are currently developing a platform with the BCLM PDOs to assess the impact of AGR2 on immune cell responses using matching peripheral blood. We expect that these findings will support further development of AGR2 blocking antibodies as a companion or alternative immunotherapy approach in metastatic breast cancer.

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If you are interested in this work and are seeking a postdoctoral position, please email: mmw178@pitt.edu

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