Tumor emboli in inflammatory breast cancer reveals macrophage-TNF-α signaling networks as targetable pathways

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Background

- Inflammatory breast cancers (IBC) are rare but highly aggressive and the leading cause of mortality in individuals with breast cancer. Instead of a palpable tumor mass generally associated with breast cancer diagnosis, IBC is marked by the presence of highly motile tumor cell clusters/emboli in the dermal tissue and lymphatic vessels.
- Hypothesis: The tumor emboli phenotype offers survival advantages during disease progression.
- Goal: Herein, using both preclinical models and patient samples, we profiled the tumor emboli to gain insights on the role of the tumor immune microenvironment in tumor emboli survival.
- Challenge: Scarcity of patient sample with tumor emboli .
- Translational relevance: The overall survival rates for patients with IBC are dismal, with nearly 30% individuals presenting with metastatic disease at diagnosis, and have not changed in the last decade. The distinct presentation of diffuse tumor cell clusters spreading collectively is postulated to provide survival benefits. Despite over 50 molecular profiling studies in the past decade, the intrinsic molecular mechanisms underlying the IBC tumor emboli phenotype remain unclear. Here, we utilize both preclinical and patient biospecimen to gain insight into the tumor emboli microenvironment and identify targets for future therapeutics. Our findings demonstrate enrichment of genes linked to chemotaxis and TNFR network of signals in the tumor cells and increased macrophage infiltration in the IBC tumor emboli microenvironment. Using a murine model, we demonstrate tumor cells treated with birinapant, a smac mimetic that enhances TNFα-mediated cell death, may be effective in targeting the tumor emboli phenotype for improved clinical outcomes.

Objective

- Developing an *in vitro* model to address sample scarcity to study transcriptome and proteome.
- Immune histochemistry study of 24 IBC patient samples to understand Tumor immune micro-environment (TiME).
- Developing a novel *in vivo* window chamber murine model to study IBC TiME.
- Utilization of the window chamber murine model for preclinical study to target immune pathway in IBC.

Methods and Results

1. Development of Tumor Emboli Organoid Culture model

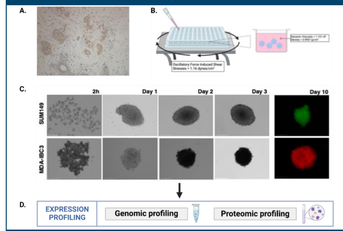


Figure 1. An in vitro method to create tumor emboli organoid to profile whole transcriptome and proteome. A. Representative image of IBC patient tumor emboli, robustly stained for XIAP. **B.** Schema of tumor emboli culture platform depicting integration of biologically relevant lymphatic viscosity and shear stress recapitulating the IBC patient TME. **C.** Representative images of IBC tumor emboli cultures maintained up to 10 days. **D.** Expression studies.

2. Tumor Emboli shows drug resistant phenotype

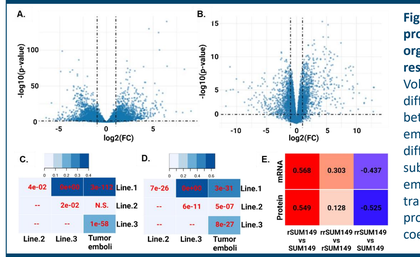


Figure 2. Whole transcriptome and proteome analysis of tumor emboli organoids reveal overlap with drug resistant IBC phenotype. A. Volcano plot of mRNA reveal differentially expressed genes between 2D cell lines and 3D emboli **B.** Volcano plot of differentially expressed proteins subpopulations between 2D and 3D emboli. **C** and **D.** Jacard index of transcriptomic and proteomic profile **E.** Calculation of correlation coefficient.

3. Tumor Emboli are highly immunogenic

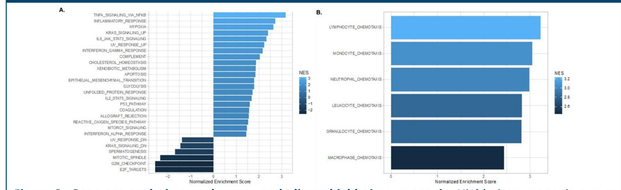


Figure 3: Gene set analysis reveal tumor emboli are highly immunogenic. Highly immunogenic tumor embolies are hallmarked by (A) TNFα:NFκB signaling axis overexpression and (B) upregulation of chemotaxis of immune infiltrates, including lymphocytes, monocytes, neutrophils, leukocytes, granulocytes, and macrophages.

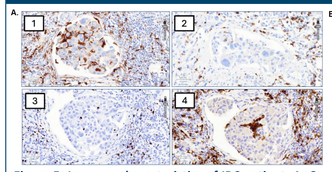
Methods and Results

4. Development of Spatial Immunophenotyping



Figure 4: Spatial IHC. A. 1. After the staining, slides are digitalized. 2. Tumor and emboli regions are annotated 3. Slides are analyzed using image analysis algorithms in Visiopharm®.

5. Immunohistochemistry shows immune cell infiltration



	RMA (E) %	RMA (IC) %	Ratio RMA E/IC	Correlation (ρ & P-value)
CD8	0.08 (0 – 5.70)	1.08 (0.06 – 5.86)	0.17 (0 – 3.96)	0.43 *P*= 0.10
CD163	0.77 (0.03 – 5.60)	0.83 (0.01 – 3.69)	0.74 (0.03 – 5.48)	0.44 *P*= 0.07
CD79α	0.04 (0.01 – 10.75)	0.57 (0.06 – 6.98)	0.11 (0.06 – 3.80)	**0.70** ***P*= 0.01**
FOXP3	0.01 (0 – 0.61)	0.09 (0.01 – 0.61)	0.16 (0 – 7.32)	**0.64** ***P*= 0.01**

Figure 5: Immune characteristics of IBC patient. A. Carcinoma cells and inflammatory cells form cohesive homotypic and heterotypic multicellular aggregates. Staining for (1) CD163 , (2) CD8, (3) FOXP3 and (4) CD79α. **B.** Cohesive tumor emboli within lymphatic vessels are often considered to be a hallmark of IBC. In 24 IBC patients examined the immune infiltrate in these emboli. We report the median RMA in the invasive carcinoma (IC), the median RMA in the emboli (E), the IC/E ratio for RMA and the correlation between the RMA in IC and E.

6. Relation of Tumor emboli and clinical outcome in patient

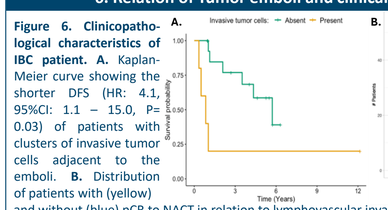


Figure 6. Clinicopathological characteristics of IBC patient. A. Kaplan-Meier curve showing the shorter DFS (HR: 4.1, 95%CI: 1.1 – 15.0, P= 0.03) of patients with clusters of invasive tumor cells adjacent to the emboli. **B.** Distribution of patients with (yellow) and without (blue) pCR to NACT in relation to lymphovascular invasion.

7. Development of in vivo murine model

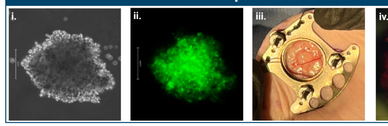


Figure 7. Elastration showing position of the window chamber with tumor emboli implanted in the dorsal skin of the CXCR mice.

Materials and Results

8. Active macrophage infiltration occurs at TiME

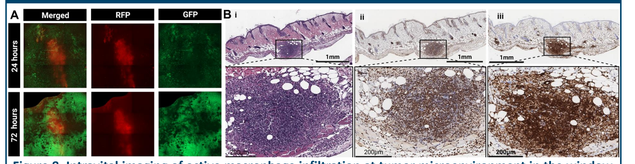


Figure 8. Intravital imaging of active macrophage infiltration at tumor microenvironment in the window chamber murine model and immune characteristics murine xenograft samples. A. Representative optical sectioning fluorescence images showing tumor cells (RFP, red) and macrophages (GFP, green) at 24 and 72 hours. **B.** Immunohistochemical analysis of implanted tumor emboli for **i.** H and E, **ii.** Ki67 and **iii.** F480 which recapitulate the immune microenvironment of IBC with enlarged view as inset.

TNFα and XIAP cross talk

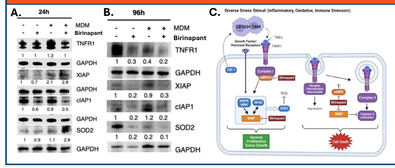


Figure 9. Birinapant inhibits TNFR1 signaling, XIAP and SOD2 expression in a TAM-simulated co-culture model.[2]. Western blot analysis of indicated targets in SUM149 cells treated with control media or with MDM-CM (± 1 μM Birinapant) for 24hr (A) or 96 h (B). C. Schema of pan-IAP antagonism in TNFR1 pathway.

Drug Development

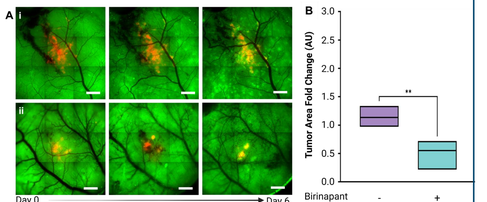


Figure 10: Effect of Birinapant treatment on implanted IBC tumor in Murine model with eGFP labeled Macrophage (CXC3R1-GFP) using Window Chamber technique. A. With Birinapant treatment (ii) the phenotype of tumor-cell cluster (in red) is changed(more compact) over the time course of 144 hrs compared to control (i) without changing Macrophage (in green) infiltration in both control and treatment. **B.** Quantification of change in tumor area upon Birinapant treatment (n=8).

Conclusion

- IBC tumor emboli contribute to TAM infiltration in TiME, driving pro-survival TNFα-NFκB-XIAP signaling leading to drug resistance.
- Birinapant is identified as a potential therapeutic agent to disrupt this signaling axis.

References

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