Irradiated Extracellular Matrix Effects on Breast Cancer Cell Invasion

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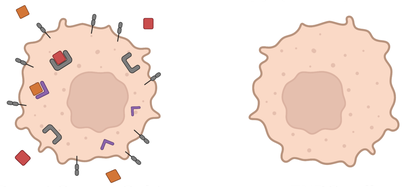
Motivation

Triple Negative Breast Cancer (TNBC):

- TNBC demonstrates relatively **high rate of locoregional recurrence** after radiation therapy (13.5%)[1]



Other Breast Cancer Subtypes | TNBC cell

Figure 1: TNBC cells lack HER2 over-expression and targetable progesterone and estrogen hormone receptors, **limiting targeted treatment options such as immunotherapy.**

Circulating Breast Cancer Cell Invasion:

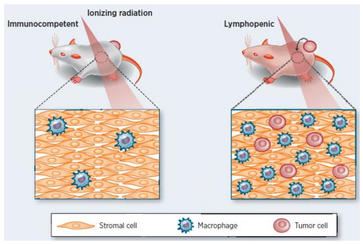


Image from Rafat et al. Cancer Research. 2018 [2]

- Lack of lymphocytes encourages macrophage infiltration tumor cell infiltration, indicating lymphocytes play a role in preventing tumor recurrence[2]
- **Cellular mechanisms behind increased invasion are unknown**

Significance of The Extracellular Matrix (ECM):

- Focal adhesions are promoted by ECM stiffening, which mediates cell to cell interactions[3]
- Radiation induces fibrosis in the tumor microenvironment by inducing fibrosis, leading to changes tumor progression

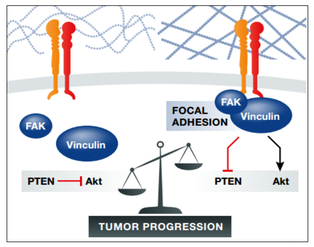


Image from Pickup et al. EMBO Reports 2018 [3]

Hypothesis

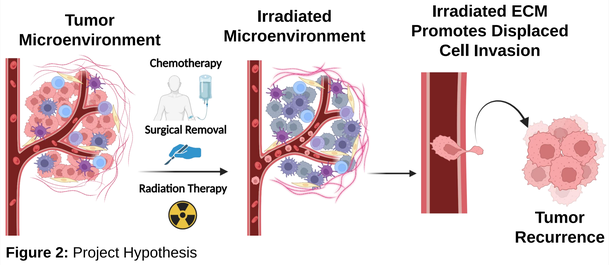


Figure 2: Project Hypothesis

Changes in the ECM induced by radiation therapy contribute to TNBC recurrence by promoting tumor cell invasion.

Extracellular Matrix (ECM) Hydrogel Formation

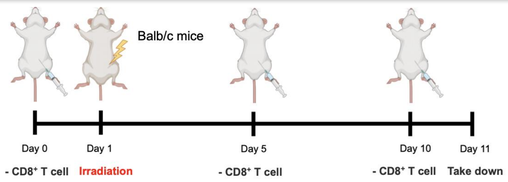


Figure 3: Timeline of Balb/c **mice *in vivo*** irradiation. Depletion–CD8⁺ T cell was completed to represent mice with lymphopenia.

- Mammary fat pads (MFPs) were extracted from Balb/c mice, decellularized to ECM components and formed into hydrogels
- Murine breast cancer cells (4T1 cell line) were embedded into hydrogels and stained with various antibodies
- Invasion quantified using fluorescence microscopy

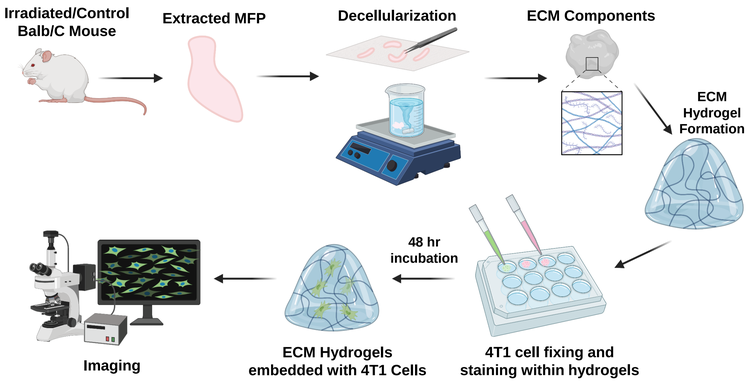


Figure 4: ECM hydrogel formation process.

Cortactin/F-Actin Colocalization

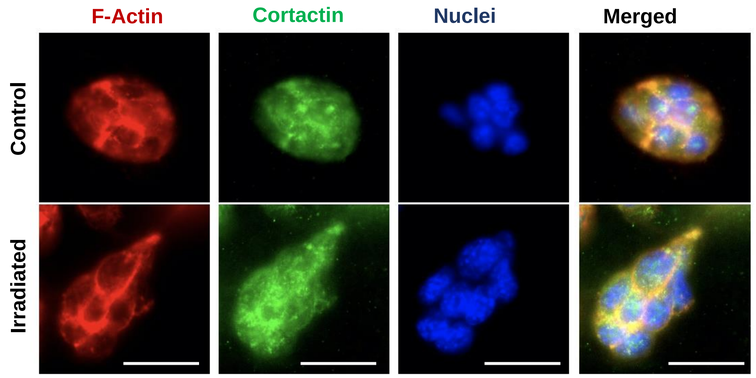


Figure 5: Representative images of 4T1 cells embedded in ECM hydrogels**.** Nuclei (blue) were used to quantify cell count. Yellow areas show **colocalization of cortactin (green) and F-actin fibers (red), associated with increased invasion**.

Scale bar represents 20 μm

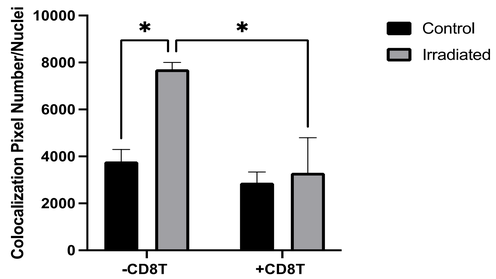


Figure 6: Colocalization was quantified by calculating the pixel overlay number for F-Actin and cortactin channels and dividing by nuclei count. A **significant increase in colocalization is shown for irradiated hydrogels derived from –CD8T cell depleted mice.**

**p<0.05, error bars represent SEM*

E-cadherin and Vimentin Expression

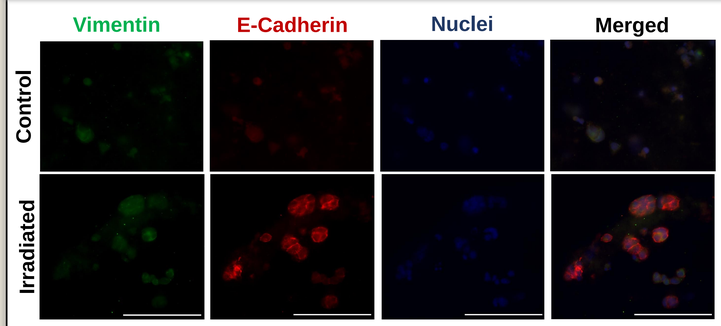


Figure 7: The epithelial-mesenchymal transition process is characterized by increased vimentin expression and decreased E-Cadherin expression, leading to increased cell invasion.[4] 4T1 cells express higher levels of vimentin when seeded into irradiated ECM microenvironments, however, increases in E-cadherin were also noted.

Scale bar represents 100 μm

MFP Elastin Staining

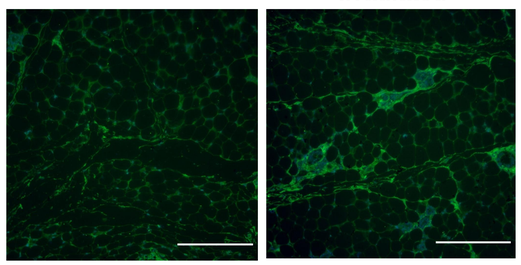


Figure 8: Immunofluorescence staining of elastin for irradiated and control ECM hydrogels from CD8+T cell depleted mice.

Scale bar represents 200 μm

Invasion Assay

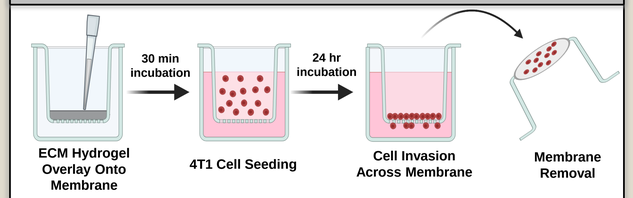


Figure 9: 4T1 cells were seeded above a permeable membrane with either irradiated or control ECM hydrogels. **Increased migration across the membrane represents increased cancer cell invasion in that microenvironment.**

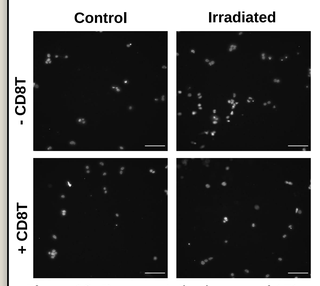


Figure 10: Representative images of 4T1 cell invasion across membrane. Cell number was quantified through counting of cell nuclei (stained via NucBlue Mountant media).

Scale bar represents 100 μm

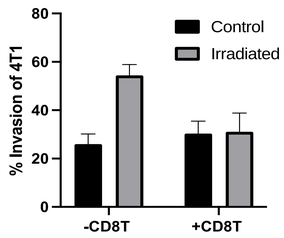


Figure 11: Nuclei counts show a increase in trend for 4T1 cell % invasion for CD8+T cell depleted irradiated ECM environments. Significant changes were not found in immunocompetent microenvironments.

Conclusions & Future Work

4T1 cell invasion properties observed:

- F-actin/cortactin colocalization and invasion assay quantification indicate **4T1 cells experienced increased invasiveness in irradiated ECM microenvironments derived from CD8+T cell depleted Balb/c mice**
- General trend of higher vimentin and E-cadherin expression in irradiated ECM microenvironments
  - Limited by lack of quantitative analysis

Future work:

- Developing methodology for quantifying expression of vimentin and E-cadherin in cells
- Expand mouse model to include bone marrow-derived macrophages to understand differences in 4T1 cell invasion for immunocompetent vs lymphopenic mice
- Determine changes in other ECM components after radiation to examine immune response effects

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Figures 1, 2, 3, 4, 11 created with BioRender.com

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