Evaluating the Impact of Immune Cell Infiltration in Therapy-Induced Breast Cancer Recurrence

Research Plan

Breast cancer remains a problem of global concern. Local recurrence rates are high for triple negative breast cancer (TNBC) patients despite aggressive surgical, radiation, and chemotherapeutic intervention (*1*). Recurrence is typically thought to occur due to the persistence of tumor cells in the irradiated surgical bed; however, recent studies suggest that recurrence may be facilitated by circulating tumor cells (CTCs) (*2*). We have determined that radiation enhances the migration of CTCs in a preclinical breast cancer model (*3, 4*). In addition, clinical data suggest that local recurrence following radiation therapy (RT) correlates with lymphopenia or abnormally low systemic lymphocyte counts (ALC) (*5*). In breast cancer models of lymphopenia, local recurrence following RT is mediated by excess macrophage infiltration into irradiated tissues Macrophage recruitment is associated with changes in extracellular matrix (ECM) deposition and metabolic regulation. The effect of these radiation-induced infiltrating macrophages on ECM remodeling and tissue metabolism is unknown. We hypothesize that tissue damage and the subsequent immune cell infiltration following RT promotes local recurrence. *The research described in this proposal will have considerable implications for the women undergoing treatment for breast cancer each year globally.* The premise that therapies themselves may contribute to cancer recurrence has not been well-explored and has the potential to challenge current paradigms about breast cancer treatment.

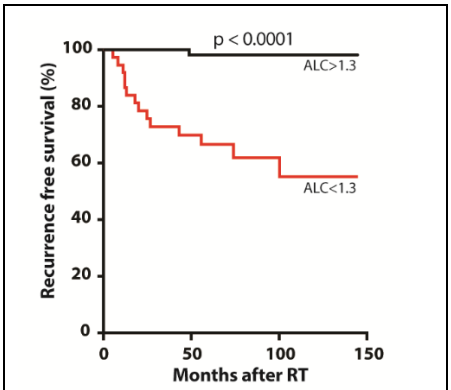

Figure 1. Lymphopenia following RT promotes recurrence in TNBC patients. Kaplan-Meier analysis of recurrence free survival in TNBC patients based on ALC (Lymphopenic ALC<1.3). Adapted from (*5*).

Preliminary Data

Lymphopenia correlates with local recurrence in TNBC patients

The impact of radiation on local recurrence in a lymphopenic setting has largely been unstudied. We previously evaluated patients with TNBC for locoregional recurrence, which was defined as recurrence in the ipsilateral breast, chest wall, or ipsilateral draining lymph nodes. ALCs for all patients were analyzed following RT, and cumulative recurrence-free survival was determined using the Kaplan–Meier method with univariate comparisons between groups using the log-rank test (*6, 7*). We determined that lymphopenia following RT is associated with local recurrence in TNBC patients (**Figure 1**).

Radiation-induced tumor cell recruitment

We studied the impact of radiation on CTC recruitment in murine TNBC models. We found that RT enhances recruitment of tumor cells from the circulation in the absence of functional CD8+ T cells. While RT did not cause tumor cell recruitment to mammary fat pads (MFPs) in immunocompetent mice, we showed that irradiation of MFPs increases the recruitment of mouse mammary carcinoma cells in immunocompromised mice (**Figure 2**). We also observed that F4/80+ macrophage infiltration precedes tumor cell recruitment and is significantly enhanced following irradiation upon CD8+ T cell depletion, provoking our hypothesis that cancer therapies may influence the migration of CTCs through altering the immune response (*5*).

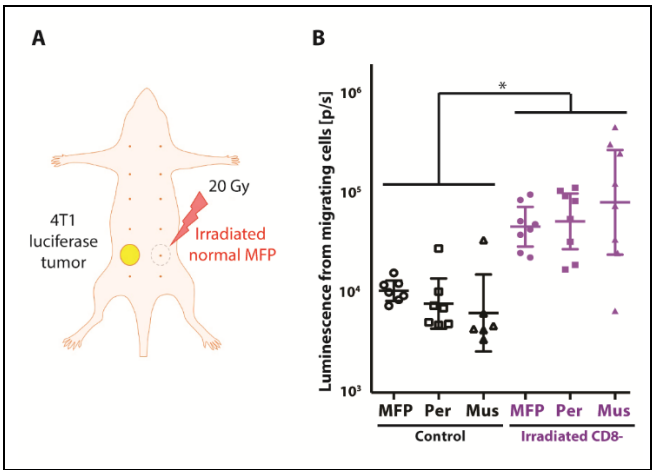

Figure 2. Irradiation of normal tissues promotes tumor cell migration *in vivo* in immunocompromised Balb/C mice. (A) Experimental schematic. (B) Tumor cell migration following RT in the 4T1 model as determined by BLI (n = 7, control; n = 8, irradiated). Statistical significance was determined by ANOVA analysis with *p<0.05. Error bars show the 95% confidence limit. Adapted from (*5*).

ECM changes in irradiated MFPs

To determine the contribution of radiation of normal tissues in providing an environment conducive to tumor cell homing and attraction, we will test the hypothesis that the changes in the ECM due to interventional therapies will alter tumor cell migratory patterns. We used scanning electron microscopy (SEM) to visualize changes in the MFP following radiation damage (**Figure 3**). Radiation not only caused changes in the uniformity of adipocyte size and shape but also enhanced ECM and collagen deposition and fiber disorder. We are interested in continuing these studies to track the MFP changes over time under different immune conditions. We will directly evaluate ECM composition changes to evaluate how these structural and biochemical

properties influence tumor cell recruitment and retention after RT. We will fabricate treatment-specific hydrogels from decellularized MFPs to determine tumor and stromal cell interactions with the damaged tissue ECM.

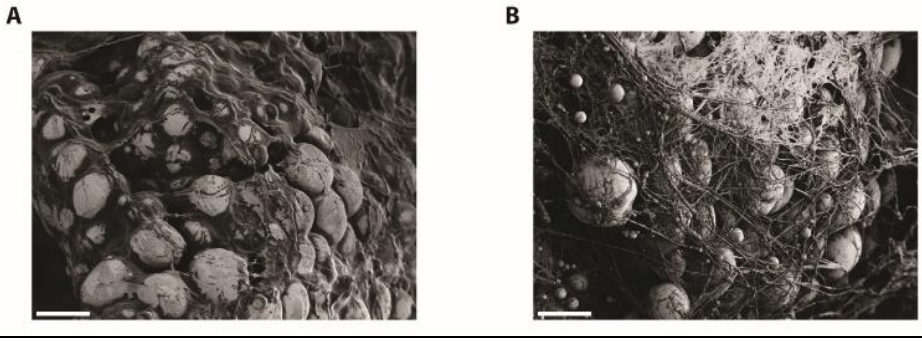

Figure 3. Visualizing tissue properties following irradiation of MFPs shows an increase in ECM deposition. Scanning electron microscopy was used to visualize the MFP in (A) unirradiated and (B) irradiated mouse tissues. Scale bar is 50 μm.

Specific Aims

In this study, we will characterize how the ECM and metabolism modulate cancer cell retention and proliferation to gain insight into local recurrence after therapy. The **goal** of this project is to establish the role that the damaged tissue microenvironment plays in modulating relapse after therapy, which will be explored in the following specific aims (**Figure 4**).

Aim 1: To study the role of wound healing from radiation and subsequent immune cell infiltration on tumor cell recruitment and ECM properties. In this aim, we propose to test the hypothesis that wounding normal tissues through radiation modifies the microenvironment to allow for tumor cell recruitment under lymphopenic conditions. Our preliminary data indicate that tumor cell recruitment to irradiated tissues is influenced by macrophage infiltration. CTCs will be tracked following radiation using bioluminescence imaging. As we have shown that macrophage infiltration is significantly enhanced following radiation damage in the absence of CD8+ T cells, we will determine the effect of wound healing from radiation on the infiltration of other immune cells, including myeloid derived suppressor cells and dendritic cells, in immunocompetent and immunocompromised mice. We will characterize ECM composition changes following RT using Raman spectroscopy since excess macrophage infiltration may induce ECM remodeling. MFPs damaged by radiation in immunocompromised and immunocompetent mice will be decellularized and used to form treatment-specific hydrogels to study tumor cell proliferation and invasion.

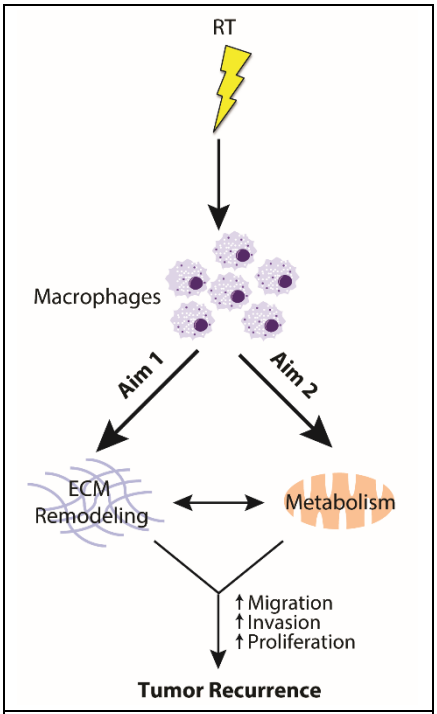

Figure 4. Aims schematic. This proposal aims to determine the role of wound healing from radiation therapy (RT) on macrophage infiltration and subsequent tumor recurrence.

Aim 2: To determine the importance of metabolic changes due to tissue damage on tumor cell recruitment. We hypothesize that tissue metabolism is dependent on both wound healing mechanisms and immune function. As changes in lipid metabolism have been shown to influence cell-cell communication, we will analyze variations in lipid droplet formation and fusion in immune and stromal cells within the MFP damaged by RT. Since it is known that macrophages can alter their lipid profile in response to microenvironmental cues, we are interested in determining how tissue damage impacts energy metabolism through measurements of oxygen consumption and extracellular acidification rates in adipocytes and infiltrating stromal and immune cells.

Completion of the above experimental plan will address the *significant and currently unmet need* of characterizing the tissue microenvironment to evaluate the conditions that induce local recurrence following therapy. Taken together, this work will advance the understanding of how matrix remodeling and metabolic changes impact tumor cell recruitment after therapy and will lead to new insights into specific, targetable mechanisms for more effective therapies to combat local recurrence.

References

1. Voduc KD et al. *J Clin Oncol* **2010**;28:1684-91.
2. Kim MY et al. *Cell* **2009**;139:1315-26.
3. Vilalta M et al. *Clin Exp Metastasis* **2018**;35:247-54.
4. Vilalta M et al. *Cell Rep* **2014**;8:402-9.
5. Rafat M et al. *Cancer Res* **2018**;78:4241-52.

Summary Budget

Personnel: PI, 5% effort: $10,000/year; Graduate Student, 50%effort each: $20,000/year.
Supplies: $20,500/year is requested for supplies and consumables.
Other: $12,000/year is requested for core facility costs.

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Lay Summary

Breast cancer poses a major health risk: in the United States, over 230,000 new cases of breast cancer were diagnosed, and approximately 40,000 women died of the disease in 2018 alone. Radiation therapy is crucial to most breast cancer patients because it is employed to eliminate any tumor cells remaining after surgery and chemotherapy. However, studies show that the relapse rate for patients after this treatment can be as high as 20%, and the causes of relapse require further study. Our previous work demonstrated that some tumor cells can be attracted to areas exposed to radiation if patients cannot recover a specific cancer-fighting immune cell after therapy. We are therefore interested in determining why tumor cells travel to treated areas and how to prevent recurrence. To understand why these irradiated, damaged tissues recruit tumor cells, we will study tissue chemical composition changes and how cells communicate with each other to help tumor cells thrive in damaged areas. By tracking migrating tumor and immune cells and exploring the changes in tumor surroundings after therapy, this research could help in developing innovative therapies that may increase the survival of breast cancer patients. Overall, this research will be crucial in determining what type of environment is conducive to tumor recurrence and re-growth. By evaluating how treatments themselves affect disease progression, this project will advance the knowledge of the factors that contribute to cancer relapse. This research will accelerate the discovery of new treatments and has the potential to increase breast cancer patient survival.