Investigating the Effect of the Tumor Microenvironment on Metastatic Progression using Micro and Nano-Scale Tools

CNF Project Number: 2912-20

Principal Investigator(s): Claudia Fischbach

User(s): Matthew Tan, Niaa Jenkins-Johnston, Nicole Sempertegui

Affiliation(s): Biomedical Engineering, Cornell University

Primary Source(s) of Research Funding: 2021 Cornell NanoScale Facility REU Program National Science Foundation Grant No. NNCI-2025233, Stem Cell Research Training Fellowship Stem Cell Program of Cornell University, the Center on the Physics of Cancer Metabolism National Cancer Institute Award Number 1U54CA210184-01

Contact: cf99@cornell.edu, mlt239@cornell.edu, noj4@cornell.edu, nds68@cornell.edu

Primary CNF Tools Used: ABM Contact Aligner, Heidelberg Mask Writer - DWL2000, Hamatech 9000, Malvern NS300 NanoSight

Abstract:

Breast cancer mortality is driven by metastasis, where cancer cells disseminate from the primary tumor to seed distant tissues. During the metastatic cascade, cancer cells interact with their microenvironment consisting of extracellular matrix including collagen and other cell types including endothelial cells in blood vessels and mesenchymal stromal cells (MSCs) in the bone. Cancer cells may interact locally or from distant sites through mechanisms such as soluble factor and extracellular vesicle (EV) signaling. In this study, CNF tools were used to investigate the two stages in metastasis: early invasion towards blood vessels and EV-mediated formation of a pre-metastatic niche. For the former, we developed a microfluidic model of the perivascular niche and found that ECs stimulate breast cancer invasion into collagen, and that an EC-coated micro channel exhibits a distinct diffusion profile from a channel without ECs. For the latter, we've been able to isolate and characterize EVs from two breast cancer cell lines. Future work will continue to use the microfluidic model to investigate the mechanisms by which ECs influence cancer invasion and apply EVs to MSCs on a bone-mimetic model system to investigate how cancer cells can influence the formation of a pre-metastatic niche.

Summary of Research:

Introduction. Breast cancer is the second leading cause of cancer-related death for women in the United States [1]. Mortality in breast cancer is driven by metastasis, where tumor cells disseminate from the primary tumor and spread to distant tissues. During this process, tumor cells become invasive and move towards blood vessels, where they will enter the circulation and seed onto distance sites such as the bones. Tumor cells that proceed through the metastatic cascade encounter a changing microenvironment consisting of extracellular matrix (ECM) such as collagen and other cell types, including endothelial cells (ECs) and mesenchymal stromal cells (MSCs) [2]. These cells are known to participate in reciprocal signaling with tumor cells to influence tumorigenesis through the exchange of soluble factors [2,3].

Incidentally, tumor cells may even be able to prime future metastatic sites through the release of extracellular vesicles (EVs) that enable long distance transport of cell-derived cargo [4]. However, the mechanisms by which soluble factor and EV signaling influence tumor cell invasion and the development of a pro-tumorigenic microenvironment remain unclear due to the lack of models that enable systematic study. To this end, we have used the expertise at the CNF to investigate two key steps in the metastatic cascade: initial invasion towards ECs in blood vessels, and EV-mediated formation of the pre-metastatic niche.

Regulation of Breast Cancer Invasion Using a Microfluidic Model of the Perivascular Niche. In early invasion, tumor cells initially invade towards blood vessels, responding to metabolic gradients from the vessels and signaling gradients from ECs. Using SU-8 photolithography in conjunction with the ABM Contact Aligner and a photomask generated by the Heidelberg DWL2000, we have created a dual-channel microfluidic devices that enables co-culture of breast cancer cells and ECs encapsulated in a 3D collagen matrix. In this system,

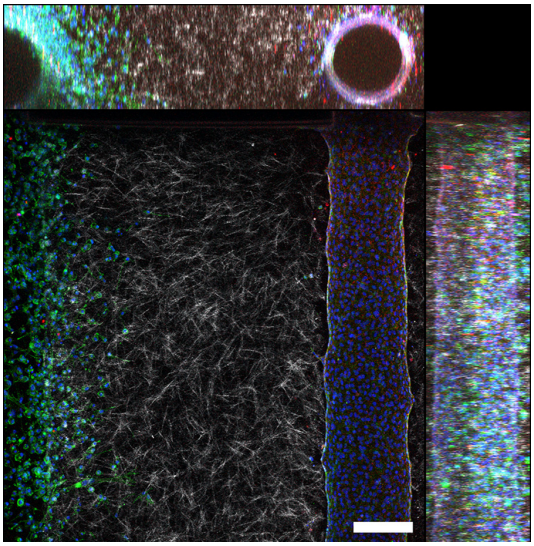

Figure 1: Confocal microscopy projection of breast cancer cells stained with invading into collagen in response to an endothelial cell channel stained with CD31 (red). DAPI was used to stain nuclei (blue), phalloidin was used to stain f-actin (green), and confocal reflectance was used to visualize collagen fibers (white). Scale bar: 200 µm.

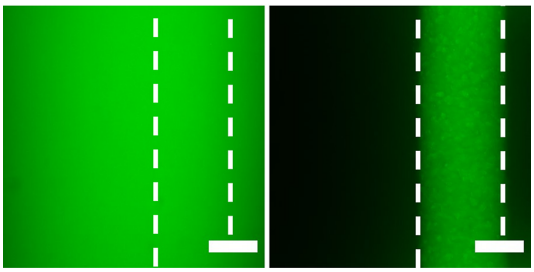

Figure 2: Diffusion of fluorescein (FITC) from a non-human umbilical vein endothelial cell (HUVEC) channel and a HUVEC coated channel. Scale bar: 200 µm.

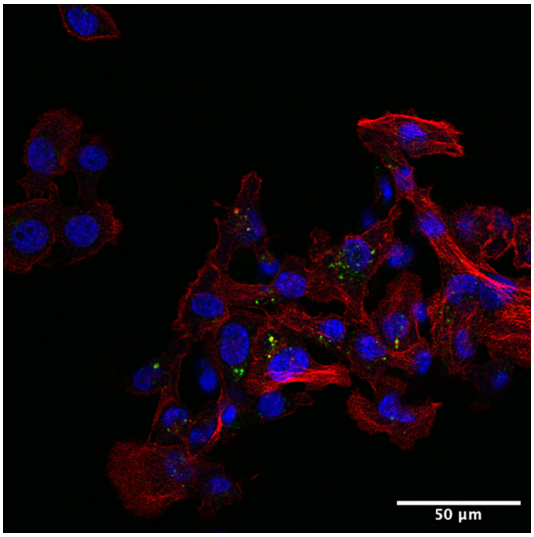

Figure 3: Extracellular vesicles labeled with a DiO lipophilic dye (green), MCF10ACA1a cells labeled with DAPI for nuclei (blue) and phalloidin for f-actin (red). Scale bar: 50 µm.

we found that the presence of ECs stimulated cancer invasion into a collagen hydrogel (Figure 1). Additionally, using fluorescent molecule diffusion studies, we found that an EC-coated channel restricted diffusion of molecules within in the channel compared to a channel without ECs (Figure 2).

Isolation and Characterization of Breast Cancer-Derived Extracellular Vesicles. Prior to arriving at distant metastatic sites, tumor cells can release soluble factors and extracellular vesicles (EVs) into the circulation to prime the microenvironment of distant target organs for subsequent development of organotropic metastasis. While soluble factor signaling plays a demonstrated role in cancer, EVs are gaining appreciation as stable vehicles of cell-derived cargo contributing to tumorigenesis and pre-metastatic niche (PMN) formation. Thus far, we have successfully isolated and characterized EVs from MDA-MB-231s and MCF10CA1a breast cancer cells using the Malvern NS300 NanoSight (Figure 3). Additionally, we have started to investigate differences in cell adhesion between MSCs cultured in the presence or absence of EVs that bind to the bone matrix.

Conclusions and Future Steps:

In this project we were able to develop tools and pipelines to study the breast cancer metastatic cascade. We were able to successfully fabricate and culture a 3D microfluidic tumor-perivascular niche model. Future work using this device will be to uncover the metabolic and mechanical mechanisms by which ECs influence breast cancer invasion. We were also able to isolate and characterize breast cancer derived ECs. Future work will assess markers of MSC behavior in the presence of EVs in a biomimetic mineralized bone scaffold. This will include investigating differences in differentiation fate, proliferation and cytokine secretion. Finally, we will examine whether these changes in MSC phenotype in turn affect metastatic breast cancer cell outgrowth.

Acknowledgements:

The work described was supported by the Center on the Physics of Cancer Metabolism through Award Number 1U54CA210184-01 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

References:

[1] Siegel, R. L., Miller, K. D., Fuchs, H. E., and Jemal, A. Cancer Statistics, 2021. CA. Cancer J. Clin. 71, 7-33 (2021).

[2] Tan, M. L., Ling, L., and Fischbach, C. Engineering strategies to capture the biological and biophysical tumor microenvironment *in vitro*. Adv. Drug Deliv. Rev. 176, 113852 (2021).

[3] Zheng, P., and Li, W. Crosstalk Between Mesenchymal Stromal Cells and Tumor-Associated Macrophages in Gastric Cancer. Front. Oncol. 10, 1-9 (2020).

[4] Peinado, H., et al. Pre-metastatic niches: organ-specific homes for metastases. Nat. Rev. Cancer 17, 302-317 (2017).