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, Nicole Sempertegui

Affiliation(s): Biomedical Engineering, Cornell University

Primary Source(s) of Research Funding: Stem Cell Research Training Fellowship, Stem Cell Program of Cornell; Center on the Physics of Cancer Metabolism Award Number 1U54CA210184-01, National Cancer Institute

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

Primary CNF Tools Used: ABM Contact Aligner, Heidelberg 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 such as endothelial cells in blood vessels and macrophages in the bone. Cancer cells may interact locally with these cells in the primary tumor microenvironment or from distant sites in the body through soluble factor signaling. In this study, CNF tools were used to investigate the two stages in metastasis: early invasion of tumor cells towards blood vessels and tumor cell invasion into the bone to form 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 microchannel exhibits a distinct diffusion profile from a channel without ECs. For the latter, the same microfluidic model was modified to incorporate collagen mineralization to mimic bone ECM. Using this model, we have shown that tumor cell invasion is inhibited when co-cultured with macrophages seeded in a mineralized microchannel. Future work will continue to use the microfluidic model to investigate the mechanisms by which ECs influence cancer invasion and how mineralized collagen affects cancer cells and 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 bone. 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 macrophages [2]. These cells are known to participate in reciprocal signaling with tumor cells to influence tumorigenesis through the exchange of soluble factors [2,3]. However, the mechanisms by which soluble factor 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 later stage invasion into the bone 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, 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).

Effects of Mineralized Collagen on Breast Cancer Cell Invasion Using a Microfluidic Model of the Bone Pre-Metastatic Niche. To colonize bone, disseminated tumor cells extravasate from the vessel and invade through the bone marrow to seed osteogenic niches [4]. Tumor cell invasion during this process is controlled by host cells including macrophages. Macrophages are particularly relevant as they not only exert immunomodulatory effects, but also have the potential to differentiate into

osteoclasts, the primary cell type driving osteolysis in bone metastasis patients. To model these interactions within a bone-like microenvironment, the microfluidic device described above was modified using the polymer-induced liquid precursor (PILP) method to include a mineralized collagen microchannel [5]. In this model, the mineralization of collagen, the primary component of bone matrix, as well as tumor cell and macrophage seeding can be selectively controlled. We have shown that macrophages promote the invasion of breast cancer cells regardless of collagen mineralization and that this effect occurs without cell-cell contact, suggesting that it was caused by soluble factors secreted from macrophages. It was also determined that collagen mineralization inhibits the ability of macrophages to promote tumor cell invasion (Figure 3).

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 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. Additionally, the 3D microfluidic device was modified to recapitulate key microenvironmental features of the bone pre-metastatic niche. These studies have shown that this model system is highly adaptable and can be modified by introducing additional cell types and/or soluble factors to probe other aspects of bone and tumor cell interactions. Future work includes will characterize the mineralized collagen and validate findings with primary murine bone marrow-derived macrophages.

Funding Acknowledgements:

Stem Cell Research Training Fellowship, by the Stem Cell Program of Cornell(NS). 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 NCI or the NIH.

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).

[5] Choi, S., et al. Intrafibrillar, bone-mimetic collagen mineralization regulates breast cancer cell adhesion and migration. Biomaterials. 198, 95-106 (2019).

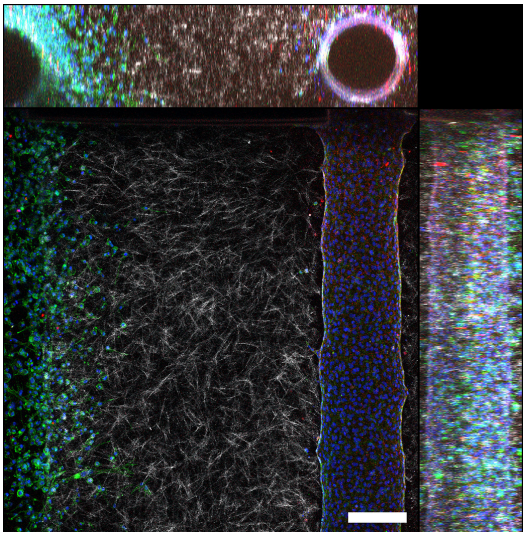

Figure 1: Confocal microscopy projection of breast cancer cells invading towards an endothelial cell channel stained with CD31 (red). DAPI was used to stain nuclei (blue), phalloidin was used to stain f-actin (green), and confocal reflectance was used to visualize collagen fibers (white). Scale bar: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

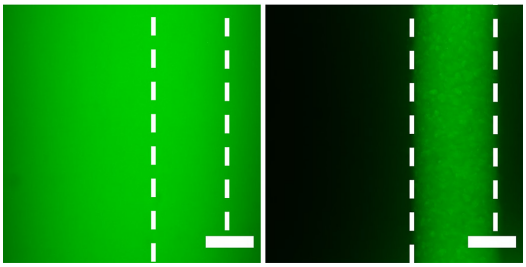

Figure 2: Diffusion of fluorescein (FITC) from a non-human umbilical vein endothelial cell (HUVEC) channel and a HUVEC coated channel. Scale bar: 200 µm.

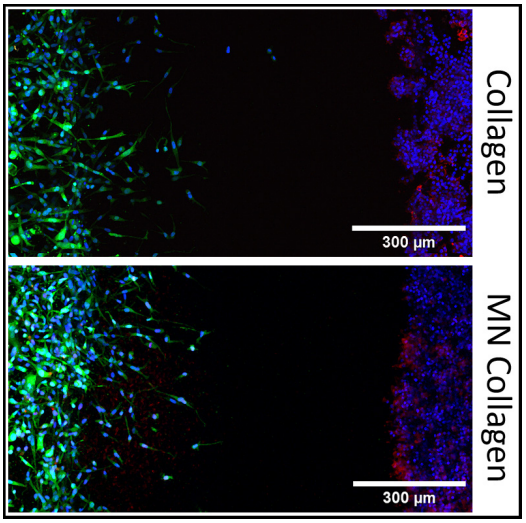

Figure 3: Fluorescent max intensity projection of non-mineralized collagen device (top) and mineralized (MN) collagen device (bottom) seeded with MDA-MB-231 breast cancer cells (green) and RAW264.7 macrophage cells (red). DAPI was used to stain nuclei (blue) Scale bar: 300 µm.