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

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CNF Principal Investigator(s): Claudia Fischbach[1]
User(s): Nicole Sempertegui[1], Garrett Beeghly[1], Jack Crowley[2]

Affiliation(s): 1. Biomedical Engineering, 2. Applied and Engineering Physics; Cornell University

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

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

Abstract:

Breast cancer is the second leading cause of cancer-related death for women in the United States [1]. Breast cancer mortality is driven by metastasis, where cancer cells disseminate from the primary tumor to seed distant tissues such as the bone. During bone metastatic disease, cancer cells interact with their microenvironment consisting of a mineralized, collagen-rich extracellular matrix and other cell types including mesenchymal stem cells (MSCs), macrophages, and osteoclasts. These cells are known to participate in reciprocal signaling with cancer cells to influence tumorigenesis through the exchange of soluble factors [2,3] and extracellular vesicles (EV). 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, CNF tools were used to investigate various stages in metastasis: EV-mediated formation of a pre-metastatic niche in bone and tumor cell invasion into bone. For pre-metastatic niche development studies, we leveraged EVs derived from breast cancer cells and bone-mimetic engineered substrates to investigate how EV binding to bone is regulated by components of their glycocalyx (sugar-coating on the outside of EVs) and matrix mineral content. For invasion studies into the bone, we developed a microfluidic model of the bone microenvironment with mineralized collagen microchannels and have shown that tumor cell invasion is inhibited when co-cultured with macrophages and osteoclasts seeded in a mineralized microchannel.

Summary of Research:

Isolation of Breast Cancer-Derived Extracellular Vesicles and Interactions with Bone Matrix. Prior to arriving at distant metastatic sites, tumor cells can release soluble factors and extracellular vesicles (EVs) into circulation to prime the microenvironment of distant target organs for subsequent development of organotropic metastasis. EVs are gaining appreciation as stable vehicles of cell-derived cargo contributing to tumorigenesis and pre-metastatic niche (PMN) formation. In collaboration with Matt Paszek's Lab, we have successfully isolated and characterized EVs from murine 4T1s breast cancer cells using the Malvern NS300 NanoSight. Currently, we are investigating differences in 4T1 EV binding to bone-mimetic engineered substrates. These bone-mimetic models were developed at the CNF using SU-8 photolithography to create a micropatterned silicon wafer used to fabricate PDMS "microwells". These PDMS microwells are filled with a collagen type I matrix and subsequently mineralized using the polymer-induced liquid precursor (PILP) method to mimic bone extracellular matrix, as previously described [5]. Through this engineered system, we can systematically explore differences in EVs binding in the presence or absence of mineral in bone. Furthermore, we are examining how EV characteristics, such as glycocalyx composition, will affect binding to bone and whether this is dependent on matrix mineral content. Our preliminary data suggest that EV binding is increased in mineralized collagen substrates, and that removal of mucins from the glycocalyx reduces their ability to bind (Figure 1). Future work will incorporate MSCs in addition to EVs to probe the interplay between bone-resident cells, bone matrix and EVs. Overall, these studies will help unravel the mechanisms by which EVs and key components of the bone microenvironment regulate the onset of bone metastasis.

Effects of Mineralized Collagen on Breast Cancer Cell Invasion Using a Microfluidic Model of the Bone. During bone metastasis, tumor cell invasion and subsequent seeding of osteogenic niches [4] can be influenced by macrophages. Macrophages not only exert immunomodulatory effects, but also have the

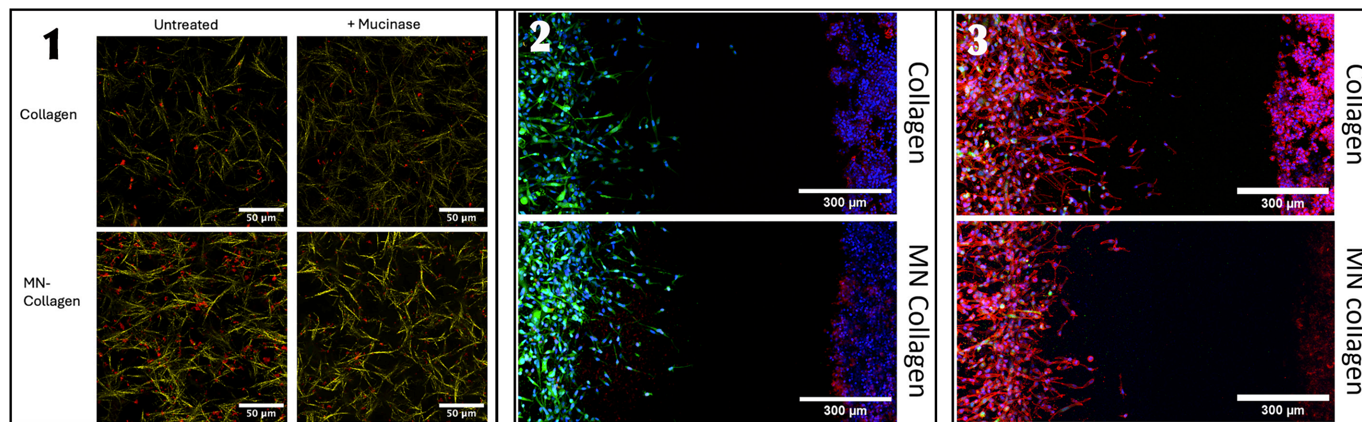

Figure 1, left: Confocal microscopy projection of 4T1 extracellular vesicles (EVs) stained with DiI lipophilic dye (red) in mineralized-collagen or collagen control microwells. Confocal reflectance was used to visualize collagen fibers (yellow). Scale bar: 50 µm.

Figure 2, middle: Fluorescent microscopy of non-mineralized collagen device (top) and mineralized (MN) collagen device (bottom) seeded with MDA-MB-231 breast cancer cells (green) and RAW264.7 macrophages (red). DAPI was used to stain nuclei (blue) Scale bar: 300 µm.

Figure 3, right: Fluorescent microscopy of non-mineralized collagen device (top) and mineralized (MN) collagen device (bottom) seeded with MDA-MB-231 breast cancer cells (green) and RAW264.7 osteoclasts. F-actin and nuclei are shown in red and blue, respectively. Scale bar: 300 µm.

potential to differentiate into osteoclasts, the primary cell type driving osteolysis in bone metastasis patients. 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 breast cancer cells and macrophages encapsulated in a 3D collagen matrix. To model these interactions within a bone-like microenvironment, the microfluidic device was modified using the PILP method to include selective and controlled collagen mineralization of the device microchannels [5]. 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. However, it was determined that collagen mineralization inhibits the ability of macrophages to promote tumor cell invasion compared to non-mineralized collagen co-culture conditions (Figure 2). Similarly, when macrophages were differentiated into osteoclasts within a mineralized collagen channel, tumor cell invasion was also inhibited compared to non-mineralized collagen devices (Figure 3). Future work includes device materials characterization and validating our findings by incorporating syngeneic breast cancer cells with primary macrophages and osteoclasts.

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