Metastatic Potential of Breast Cancer Cells is Indicated by Adhesion Strength
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Introduction: Metastasis requires cancer cells to migrate away from the primary tumor and establish secondary tumors at distal sites. Tumors are heterogeneous and only a small fraction of cells are capable of metastasizing. Migration is driven in part by interactions between cells and the surrounding extracellular matrix (ECM), and the stability and strength of cellular attachments to these matrix proteins is a key physical component of migration. We have recently shown that metastatic lines arising from epithelial tumors demonstrate lower cellular-ECM adhesion strength than their non-metastatic counterparts, but with significant heterogeneity even within metastatic cell lines¹. Using a microfluidic chamber to isolate weakly adherent cells, we now demonstrate differences in migration and invasion propensity that can correspond to heterogeneous metastatic potential.

Materials and Methods: MDA-MB231 and NIH1299 representing mammary, prostate, and lung epithelial tumors were cultured according to standard protocols. Initial population adhesion strength was characterized using the spinning-disk shear assay¹ to determine the shear stresses at which the weakest and strongest fractions of cells could be isolated. To isolate weakly adherent cells, cells were seeded at low density onto a fibronectin-coated glass slide. The slide was clamped to a polysulfone base plate with an inlet and outlet (Fig. 1A). Cells were exposed to an acute, low shear stress in low cation containing buffer that mimic stromal conditions². Fluid that exits through the outlet is spun down, and the cells are re-suspended in growth media. Cells were exposed to high shear stress, after which the adherent cells were detached with trypsin, spun down, and re-suspended in culture media. Both cell fractions were plated on 2.4 mg/mL collagen gels for migration assays, and cell migration was tracked using ImageJ.

Results and Discussion: To isolate weakly adherent cells for characterization, we designed a microfluidic chamber that would expose a plate seeded with cells to a uniform shear that is controlled by the fluid flow rate through the chamber. For MDA-MB231 cells at 5 dynes/cm², weakly-adherent cells isolated from the flow-through represented 5% of the population whereas adherent cells above 90 dynes/cm² were the 10% most strongly adherent portion of the population. Weakly adherent cells were more than 2.5-fold more motile than their strongly adherent or unselected counterparts (Fig. 1B) and were more processive based on total displacement (Fig. 1C). Increased migration propensity was maintained over time (Fig. 1D), indicating that there is stability in the phenotypic differences that result from differences in focal adhesion assembly; in low stromal cation conditions², focal adhesions are more dynamic and less assembled in metastatic lines versus non-malignant lines¹. Similar results for lung epithelial tumor cells suggest common correlations between adhesion strength, adhesion assembly, and the highest metastatic potential for tumors that represent the majority of all metastatic, solid tumors in the US.

Conclusions: Heterogeneity in cancer cell adhesion strength indicates variable metastatic potential in stromal-like conditions. Our microfluidic separation method allows us to sort metastatic cells based on this potential, which stratifies motility rates that inversely scale with adhesion strength. These data suggest the potential prognostic capacity of this assay for examining tumor cells post resection as an indicator of recurrence free survival time.