Tumor heterogeneity remains a clinical challenge in cancer as it drives differential responses of cancer cells subpopulations to various environmental cues [1]. Both mechanical cues, such as confinement, and metabolic cues, such as levels of metabolic intermediates like reactive oxygen species (ROS), have been shown to promote cell invasion and migration. [2]

**Role of heterogeneity in breast cancer cell behavior**

MDA-MB-231 triple negative breast cancer cells (MDA) were sorted into highly (MDA+) and weakly (MDA-) migratory subtypes and assessed their differential response to metabolic cues. **Figure 1 (A-B)**

- **A**)-high ROS production leads to cytotoxicity and cell death. [3]
- **B-C**) Preliminary testing of parental MDA-MB-231 in the presence of glycolysis inhibitor, 2-deoxy(D)-glucose (2DG) or oxidative phosphorylation inhibitor, antimycin A (AMA) in orange. Shift in velocity distribution indicates subpopulation in AMA treated-cells that increase speed upon oxidative phosphorylation inhibition. (n=35-45)

We aim to better understand how ROS production contributes to breast cancer cell migration, and how the dosage of inhibitors affects the behavior of migratory cells.

**Materials and Methods**

**Collagen Microtracks Fabrication**

- **A** PDMS Stamp
- **B** Collagen Microtracks
- **C** Schematic of micromolding process. Wafer is cast in polydimethylsiloxane (PDMS) and cured. (B) PDMS is peeled away and used as a stamp to mold Type I 3mg/ml collagen. [2]

**Phenotyping Cell Sorting**

To purify differentially migratory cells, parental MDA-MB-231 cells (MDA) were seeded in a transwell migration assay. 

**Figure 2 (A)**

- **A** Graphic representation of the sorting cell process into highly or weakly migratory cells. [2]

When cells are treated with a high 100 µM dose of the ROS activator, tert-butyldihydroperoxide (TBHP), significantly decreased speed in both subtypes, conflicting with previous reports. **Figure 4 (A-B)**

- **A**) Inhibiting glycolysis decreased migration speed for both MDA+ and MDA-, but more significantly for MDA+. (N=3, n=57-49) 
- **B**) Inhibiting mitochondrial respiration increased MDA+ migration but had little effect on MDA- migration (n=3+)

At low doses of ROS activation, MDA+ velocity distribution shows that some fraction of cells increase speed slightly, indicating that ROS may have different effects at low doses. The 25 µM promotes motility while 50 µM and 75 µM decreases motility.

**Future Work**

We want to test how the different dosages of inhibitors influence the ROS levels in the highly and weakly migratory populations of MDA cells and how is their velocity affected by the metabolic cues, different inhibitors, and other mechanistic approaches.

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**References**