Using a Microtrack Platform to Determine the Effect of Reactive Oxygen Species on Confined Migration

Andrea Valero[1], Qinzhe Xing[2], Reethi Padmanabhan[2], Jenna A. Mosier[2], Dr. Cynthia Reinhart-King[2]
[1]Department of Chemical and Biomedical Engineering, University of Texas at San Antonio, [2]Department of Biomedical Engineering, Vanderbilt University

Background

- 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]

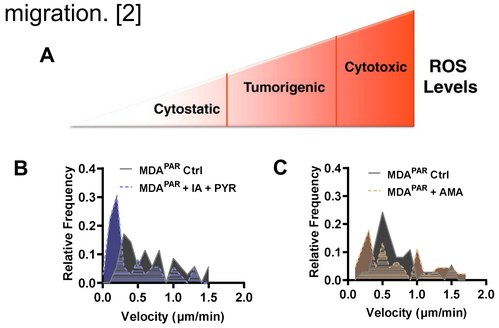


Figure 1 (A) *Higher ROS production leads to cytotoxicity and cell death [3].* ***(B-C)*** *Preliminary testing of parental MDA-MB-231 in the presence of glycolysis inhibitor, iodoacetate (IA) in purple or oxidative phosphorylation inhibitor, antimycin-A (AMA) in orange. Shift in velocity distribution indicates subpopulation in AMA treated-cells that increase speed upon oxidative phosphorylation inhibition (n=35-45) [4].*

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

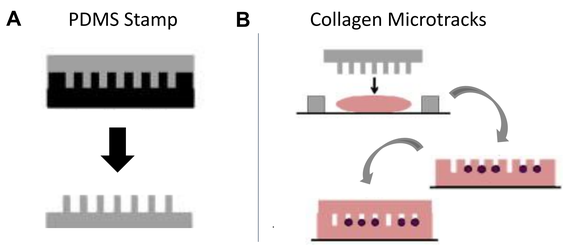
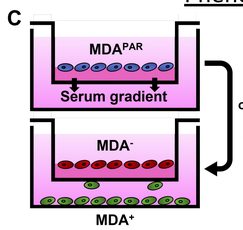


Figure 2 (A) *Schematic of micromolding process. Wafer is cast in polydimethylsilozane (PDMS) and cured.* ***(B)*** *PDMS is peeled away and used as stamp to mold Type I 3mg/ml collagen.[2]*

Phenotyping Cell Sorting



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

(C) *Graphic representation of the sorting cell process into highly or weakly migratory cells. [4]*

Preliminary Results

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.

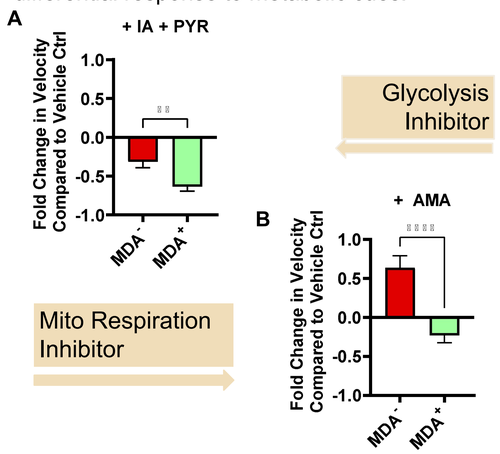


Figure 4 Preliminary Data (A) Inhibiting glycolysis decreased cell speed for boh MDA+ and MDA-, but more significantly for MDA+. (N=3+, n=34-36). **(B)** Inhibiting mitochondrial respiration increased MDA- migration but had little effect on MDA+ migration (N=3+, n=57-69); [3]. ** denotes p<0.01 **** denotes p<0.0001

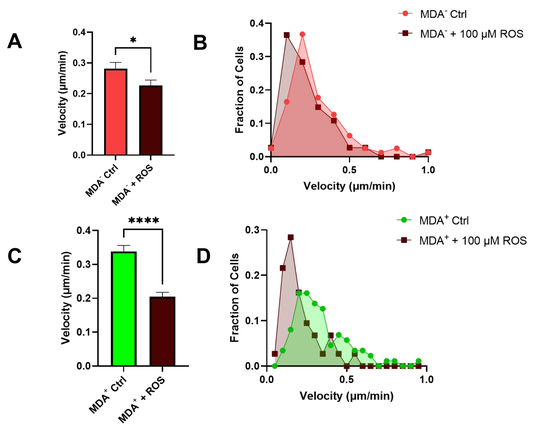


Figure 4. **(A-B)** Treatment of MDA- with high dose of ROS activator, tertbutylhydroperoxide, TBHP, results in significant decrease in migration speed and shift in velocity distribution. (**C-D**) Treatment of MDA+ with 100 μM TBHP results in significant decrease in migration speed and shift in velocity distribution [4] (N=3, n=54-87). * denotes p-value <0.05, **** denotes <0.0001.

When cells are treated with a high 100 μM dose of the ROS activator, tertbutylhydroperoxide (TBHP), significantly decreased speed in both subtypes, conflicting with previous reports.

Results

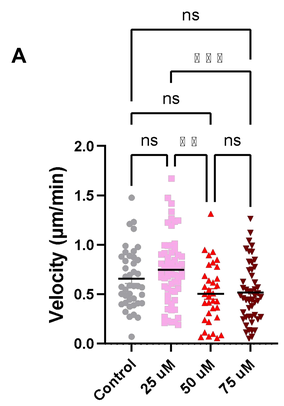


Figure 5 (A) 25 μM TBHP results in slight increase in migration speed compared to vehicle control, but significantly increase migration speed compared to 50 and 75 μM, indicating a potential biphasic response to low doses of ROS. (N=3, n=34-60) ** denotes p<0.01 **** denotes p<0.0001

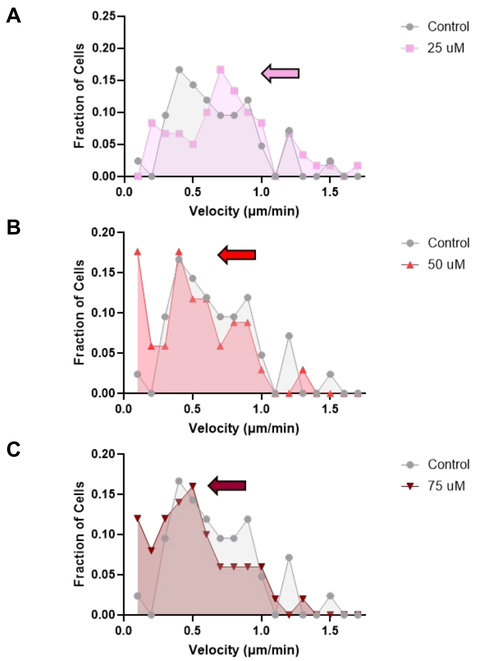


Figure 6 (A), (B), and (C) Distribution of speeds of MDA^{PAR} of 25, 50, and 75 μM doses of TBHP compared to vehicle control. Arrows indicate velocity peaks in each distribution. 50 and 75 μM doses result in leftward (decreasing) shift, suggesting a uniform response to treatment. However, the low 25 μM contains peaks on either side of control distribution. (N=3, n=34-60).

When treated with 25 μM TBHP, a fraction of MDA^{PAR} increased migration velocity, suggesting that at low doses, ROS may have differential effects on different cell subtypes.

Conclusions

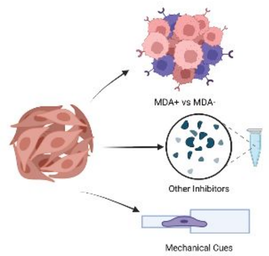
This research uses a novel 3D platform to examine the contribution and influence of ROS on breast cancer cell migration with the purpose of understanding how to target metastasis.

Previous research confirmed MDA+ and MDA- have differential responses to mitochondrial respiration inhibition. Because inhibition of this results in large production of ROS, we determined subpopulation response to increased ROS. At high doses, however, both MDA+ and MDA- significantly decreased speed, prompting us to understand how different levels of ROS change migration.

At low doses of ROS activation, MDA^{PAR} 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 demotes 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.



References

[1] El-Sayes N, Vito A, Mossman K. Tumor Heterogeneity: A Great Barrier in the Age of Cancer Immunotherapy. Cancers.2021;13(4):806. https://doi.org/10.3390/cancers13040806
[2] Petrov, Veselin & Hille, Jacques & Mueller-Roeber, Bernd & Gechev, Tsanko. (2015). ROS-mediated abiotic stress-induced programmed cell death in plants. Frontiers in plant science. 6. 69. 10.3389/fpls.2015.00069.
[3] Sullivan, Lucas & Chandel, Navdeep. (2014). Mitochondrial reactive oxygen species and cancer. Cancer & metabolism. 2. 17. 10.1186/2049-3002-2-17.
[4] Schwager, Mosier, et al. 2022, iScience, “Link Between Glucose Metabolism and EMT Drives Triple Negative Breast Cancer Migratory Heterogeneity”. In Revision.
[5] Hapach et al., 2021, Cancer Research “Phenotypic heterogeneity and metastasis of breast cancer cells”

Acknowledgements

The authors of this poster would like to acknowledge the Reinhart-King lab, VINSE, and the cleanroom staff for their help, as well as the National Science Foundation for funding via the VINSE REU grant (NSF-DMR 1852157), the NSF-GRFP to J.A.M. (DGE-1937963), the W.M. Keck Foundation, and the National Institutes of Health (GM131178)