Elucidating the Effect of Reactive Oxygen Species on Confined Cell Motility

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Migration is an essential function of many cells for the proper execution of differing physiological processes, including wound healing, proper immune response, and tissue homeostasis.[1] In addition, migration also contributes in the pathologies of diseases; in the typical progression of cancer, metastasis, the process that characterizes the dissemination of cancerous cells from a primary tumor site to the establishment of secondary tumorigenic locations, is defined by the capacity of migratory cells to effectively migrate through differing physiological environments.[2]

As the complex nature of tumors makes broad treatment nearly impossible, the prevention of metastasis could provide a way to drastically improve patient prognosis.[3, 4] To be accurately studied, the 3D microenvironment that these migratory cells move through must be replicated in vitro. The channel-like tracks that migratory cells move through range between 3 μm to 30 μm in width and height to 100 μm to 600 μm in length.[5] However, as cells move through these confining spaces, they are subject to differing levels of physical restraint and internal stresses. It has been demonstrated that confinement compromises the nuclear envelope integrity, resulting in DNA double-stranded breaks; studies have suggested that the cause of these DNA double-stranded breaks may be attributed to the diffusion of Reactive Oxygen Species (ROS) across damaged nuclear lamina during NER, but the role of ROS in confined migration remains unclear.[6, 7, 8]

Using a high-throughput PDMS-based microfluidic model which allows for live-cell imaging during migration, HT-1080 fibrosarcoma cells (cancerous) and fibroblasts (noncancerous) were cultured and seeded into these devices. Cell lines were subjected to both partially(100 μm^2) and fully (30 μm^2) confined channels, and N-acetyl cysteine (NAC), a ROS scavenger, was added to cell culture media at the commencement of each experiment to inhibit the presence of ROS. With HT-1080 cells, we observed that overall migratory speeds increased markedly in response to treatment with NAC in fully confined spaces, while no discernable increase was seen in partially confined spaces. Fibroblasts exhibited slightly increased speeds in confinement, but not significantly so.

The frequency of nuclear blebbing, or bulb like protrusions seen extending from the nuclear lamina, decreased in both cell lines after treatment with NAC in fully confined spaces, again having no effect in partial confinement. The same trend was seen when looking at migratory phenotype; the protrusive phenotype, or the more aggressive migratory phenotype, was seen in higher frequency in full confinement after treatment with NAC. Both nuclear blebbing and cell phenotype were quantified by fixing cells using paraformaldehyde and staining the cells with two fluorescent markers: Hoechst, a marker that emits blue fluorescence upon excitation by UV light after binding to Adenine-Thymine regions of nucleic DNA, and Phalloidin, a peptide which binds to F-actin filaments in cell cytoplasm and emits green fluorescence after UV stimulation.

As nuclear blebbing typically coincides with NER, we transfected HT-1080 and fibroblast cells with a fluorescent reporter which allows for visualization of localized ruptures in real time. Inhibition of ROS markedly suppressed the incidence of NER in fully confined channels, suggesting an increase in cellular ROS levels may affect nuclear lamina integrity. It has also been demonstrated that cell death in long term confinement (~3 days) is

markedly suppressed in confined channels after treatment with NAC in fibroblasts.

Our results reveal that not only do ROS play a role in confined cell migration, but their concentration can also modulate migratory cell characteristics. As ROS have been shown to hyperactivate protumorigenic effects in elevated intracellular levels like increased cancer cell proliferation and angiogenesis, ROS have also been shown to induce tumor inhibiting characteristics in high concentrations; prolonged inflated levels of ROS can induce cancer cell death.[9] Better understanding the exact effects of ROS on both cancerous and noncancerous migration is an important step in working towards improving patient care. We hope to continue to investigate the effects of ROS by using a novel fluorogenic probe, CellROX, to visualize the localization of ROS within cells. As the ROS scavenger only had an effect in fully confined channels, we hypothesize that intracellular levels of ROS will be elevated in cells moving through full confinement as compared to cells moving through partial confinement.

Statement of Research Advisor

Collins' research has delineated previously unknown conclusions involving a complicated relationship between reactive oxygen species and confined cell motility. He has independently driven all aspects of this project and his efforts will allow for more in-depth exploration into confined cell migration.
- Dr. P. Mistriotis, Chemiscal Engineering, Samuel College of Engineering

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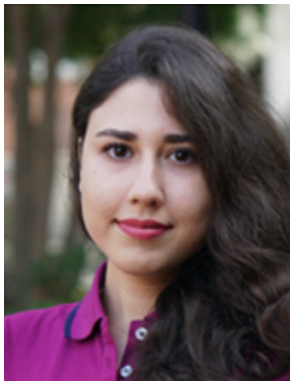
Authors Biography

Collins Keith is a senior in the department of Chemical Engineering at Auburn University. From Birmingham, Collins joined the Mistriotis lab in March of 2021, where he is conducting a project investigating the effects of Reactive Oxygen Species on confined cell migration as an undergraduate research fellow. Collins aims to pursue a M.D./Ph.D. program post-graduation



Farnaz Hemmati received her bachelor's and master's degrees in chemical engineering from the Iran University of Science and Technology (IUST). She has been a Ph.D. student in the Mistriotis Lab since January 2020, her research project focusing on uncovering the role of 3D confinement in cell behavior.

Farshad Amiri is a graduate student in the Department of Chemical Engineering at Auburn University. He received his bachelor's and master's degree in chemical engineering from the University of Tehran, Iran. Farshad joined the Mistriotis' lab in January of 2020 and is interested in combining engineering tools and concepts with advanced cell and molecular biology.

Panagiotis Mistriotis, Ph.D., is an Assistant Professor in the Department of Chemical Engineering and a Ginn Faculty Achievement Fellow in the Samuel Ginn College of Engineering at Auburn University. In his postdoctoral work, which was in part funded by an American Heart Association Postdoctoral Fellowship, he integrated cutting-edge μ-fluidics, imaging, and molecular biology techniques with mathematical modeling to uncover the underlying molecular mechanisms regulating cancer cell locomotion inside confining microenvironments.