

Chem 218: Student Exit Seminar

“Quantifying the Actin Cytoskeleton: A non-invasive image quantification tool to identify and track sub-cellular processes in real-time”

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ABSTRACT: The actin cytoskeleton is an essential component of the cell aiding in a range of biological functions from movement and maintaining the shape of the cell to transporting sub-cellular cargo. In our work, we have investigated lung cancer and pre-cancer cells to understand the importance of actin cytoskeleton in identifying and tracking sub-cellular processes. In lung cancer cells, we have identified a novel intermediate phenotype during Epithelial Mesenchymal Transition (EMT) with a unique cytoskeletal architecture based on the relative orientation of stress fibers. From localization of actin binding proteins (APBs) we have demonstrated that the intermediate and final state has predominantly different types of stress fibers. To quantify the cytoskeletal difference between these phenotypes, we have developed a method Statistical Parametrization of Cell Cytoskeletal Orientation (SPOCCO) that uses Orientational Order Parameter (OOP) as a figure of merit to track the progression of EMT. Our image quantification technique has improved throughput and is non-destructive compared to other existing techniques to track biological changes, such as mass-cytometry or RNA-sequencing. Due to the extensive inter-dependence of the cell cytoskeleton with its mechanical properties and in turn their invasiveness, we have correlated the OOP data with Young's modulus derived from AFM experiments to further validate the intermediate nature of the novel phenotype. In pre-cancer cells, it has been reported that cells derived from non-cancerous epithelial tissue samples also demonstrate enhanced invasiveness similar to mesenchymal cells. Here we have delved into the cytoskeletal architecture of this high migratory cells that demonstrated almost mesenchymal-like stress fiber structure. Using the cytoskeleton as a marker, we have identified that inhibition of the ERK-MEK pathway disrupts the cytoskeleton and affects their migratory capabilities



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Via Zoom