1638-Pos Board B530
Macrophage Migration and the Roles of WASP, Cdc42, and PI3K
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Macrophages are an important component of the innate immune response and their ability to migrate toward pathogens is critical to their success as first responders. Several proteins are known to contribute to the migration of macrophages and their roles have been studied using Boyden chambers, Dunn chambers, and micropipette pointsources. However, visualizing macrophage migration has been difficult due to their inherently strong adhesion. We have been able to study macrophage migration in two dimensions using a novel surface preparation in which PDMS coated coverslips are stamped with the extracellular matrix protein fibronectin. LR5 macrophages were individually tracked on these surfaces in the presence of CSF-1, a physiologically relevant chemokine, using time-lapse microscopy. Analysis of the trajectories of motion has allowed us to describe motility in terms of speed, persistence time, and the random motility coefficient. LR5 cells in which endogenous Cdc42 or WASp was reduced using small interfering RNA were also examined to determine the role of these proteins in migration. Our results indicate that reduction of WASp levels leads to a significant reduction in motility. The knockdown of Cdc42 leads to a reduction in the random motility coefficient and sensitivity to ligand chemistry. Our results are in contrast to other studies which have linked Cdc42 and WASp to directional sensing but have found no defect in random motility. The roles of PI3K in macrophage migration are currently being investigated. We are also beginning to study the forces macrophages produce while migrating using microfabricated post array detectors. The cell lines with reduced protein levels will also be studied on posts to determine the role of each protein in force production.

1639-Pos Board B531
The Nanomechanical Signature of Breast Cancer
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Cancer initiation and progression follow complex molecular and structural changes in the extracellular matrix and in the cellular architecture of living tissue. Yet, it remains poorly understood how the transformation from health to malignancy alters the mechanical properties of cells within the tumour microenvironment. Here we show using an indentation-type atomic force microscope (IT-AFM) that unadulterated human breast biopsies display distinct stiffness profiles. Correlative stiffness maps obtained on normal and benign tissues show uniform stiffness profiles that are characterized by a single distinct peak. In contrast, malignant tissues have a broad distribution resulting from tissue heterogeneity with a prominent low-stiffness peak representative of cancer cells. Similar findings are seen in specific stages of breast cancer in MMTV-PyMT transgenic mice. Further evidence obtained from the lungs of mice with late-stage tumours shows that migration and metastatic spreading is correlated to the low-stiffness of hypoxia-associated cancer cells. Overall, nanomechanical profiling by IT-AFM provides quantitative indicators in the clinical diagnostics of breast cancer with translational significance.

1640-Pos Board B532
Elastic Moduli of Cells undergoing Neoplastic Transformation
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We report on experiments using an Atomic Force Microscope (AFM) with a 5.3 um diameter spherical probe to measure the elastic modulus of human mammary epithelial cells (HMEC) as they undergo a neoplastic transformation from normal to immortal and finally tumorigenic. Our results suggest that normal cells demonstrate a significant difference in modulus depending on their environment, viz. whether they are isolated, on the periphery of a colony, or inside a colony. Measurements to date indicate that the cytoplasmic moduli in these 3 different environments for normal HMEC cells are 400 +/- 25 Pa, 470 +/- 37 Pa, and 710 +/- 38 Pa respectively (modulus +/- sem). We expect to report moduli under similar conditions for immortalized and tumorigenic HMEC cells. In addition, for normal HMEC cells, we observe moduli differences due to cellular structures with the nuclear modulus being significantly higher (500 +/- 25 Pa, 700 +/- 60 Pa, and 750 +/- 42 Pa respectively) than the modulus of the cytoplasm. We also plan to report on these structural differences for immortalized and tumorigenic versions of HMEC cells.
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1641-Pos Board B533
Changes in the Mechanical Properties of Cells undergoing Neoplastic Transformation
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We report on our effort to characterize the mechanical changes in cells that undergo neoplastic transformation. The cells we are studying include normal human mammary epithelial cells, as well as immortalized and tumorigenic versions of the same cells. We are studying three different aspects of cell behavior using different tools. The measurements include determining the elastic moduli of the different cells (in both the nuclear and cytoplasmic regions) using an Atomic Force Microscope (AFM) with a 5.3 um diameter spherical probe; measuring the diffusion and binding of mismatch repair proteins in the different cells using a confocal microscope to perform Fluorescence Recovery After Photobleaching (FRAP) measurements as well as Raster Imaging Correlation Spectroscopy (RICS); and finally measuring the diffusion and transport of different natural organelles such as lysosomes and peroxisomes using particle tracking microscopy. In this presentation, we plan to provide a summary of results in all three areas to date, and to put our measurements in the context of related work published by others.
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Regulation of Cell- Generated Forces during Phenotypic Reversion by a Novel Laminin Chain in 3D Culture of Human Breast Tumor Cells
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Laminins are heterotrimeric extracellular glycoproteins found in, but not confined to, basement membranes (BMs). They are important components in formation of the molecular networks of BMs as well as in cell & tissue polarity, differentiation and morphogenesis. They are secreted by cells and serve to initiate the template required for multicellular assembly. Mass spectroscopy of the isolated membrane fractions revealed a differential expression of an unexpected laminin chain where human malignant breast cells have a higher expression than that of non malignant cells in 3D lrECM. We thus asked the question what is the role of this endogenously produced laminin for 3D acinar morphogenesis. Recently, we reported that non-malignant cells in a 3D laminin-rich gel generate a centripetal force resulting in coherent angular motion (CAMo) to establish acini. On the other hand, malignant cells are randomly motile but regain the ability to rotate during 'phenotypic reversion'. We show that there is a differential deposition of this laminin in the BM in malignant tissues from human patient biopsies. Modulation of expression via shRNA reveals that malignant cells regain the ability to generate centripetal forces and thus re-enter the acinar morphogenetic program. We determine that the cells' ability to perform CAMo is in part due to both the expression and localization of this particular laminin chain. These data show the intimate dynamic reciprocity between the cells and the ECM to establish polarity and form tissue architecture

1643-Pos Board B535
Measuring Intracellular Viscoelastic Properties of Normal and Transformed Human Mammary Epithelial Cells by Tracking Organelles
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Cancerous cells display altered cytoskeletal structures from their normal counterparts, and their ability to invade other tissues demands mechanical properties that are conducive to deformation. Unlike proteins, water, and small molecules, the motions of large endogenous particles and organelles are sensitive to the mesh sizes and mechanics of the cytoskeletal network. By tracking peroxisomes, lysosomes, and other large vesicles, we measure the cytoplasmic viscoelastic properties of cells. To compare the viscoelastic properties of normal human mammary epithelial (HME) cells to those of tumorigenic, transformed HME cell lines, organelles undergoing Brownian motion within each cell type were tracked at 100 fps. The viscoelastic moduli were calculated from the mean square displacements and radii of these organelles using Mason's generalized Stokes-Einstein equation (GSE). In both normal and tumorigenic cell types, the curve for η^* shows a low frequency viscosity of approximately 10^{-1} Pa s and exhibits shear-thinning. Similarly for both types, the viscous component