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AFM Indentation Reveals Actomyosin-Based Stiffening of Metastatic Cancer Cells during Invasion into Collagen I Matrices
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Atomic force microscopy (AFM) is a widely used technique to directly probe the mechanical response of mammalian cells to external forces to determine their elasticity. To date, the majority of AFM-based studies are limited to cells that are adhered to flat substrates, however these measurements lose information regarding cell mechanobiology in a physiologically relevant 3D microenvironment. We have performed combined AFM indentation and confocal fluorescence measurements on MDA-MB-231 metastatic breast cancer cells which have either partially or fully invaded into polymerized bovine collagen I matrices. In order to interpret the raw data from the experiments to determine the cells' elastic modulus, we have developed numerous analytical and simulation techniques. A sphero-conical tip geometry to represent an AFM probe with a spherical cap transitioning to a cone is derived and applied to analyze deep indentations into the cell-collagen layer. For partially invaded cells, a generalized bonded two-layered elastic half-space model is numerically solved to assist with decoupling the mechanical response of the collagen matrix from the cell. For fully embedded cells, finite element analysis is used to simulate an AFM indentation to extract their elastic moduli. Using these techniques, we demonstrate that the elastic modulus of MDA-MB-231 cells significantly increases by ~80% as they invade into collagen compared to cells on glass and cells on top of collagen. Inhibiting ROCK decreases the rigidity of cells on a surface as well as the magnitude of stiffening during invasion into collagen. These results corroborate recent actomyosin-based rounded cell motility models in 3D and demonstrate the ability of AFM to study cell mechanics in tissue-like environments.

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Cytoskeletal Forces during T Cell Activation
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T cell activation is critical for the adaptive immune response in the body. The binding of the T cell receptor (TCR) with antigen on the surface of antigen presenting cells (APC) triggers signaling cascades and cell spreading. Physical forces exerted through the TCR have been shown to induce signaling events, but the origin of how these forces are generated and maintained is unknown. Here, we use traction force microscopy to measure the forces exerted by Jurkat T cells during TCR activation. We used anti-CD3 coated elastic polyacrylamide gels to stimulate Jurkat T cells and measured the spatially resolved traction stress map exerted by these cells as they were activated. Perturbation experiments revealed that stresses were largely generated by actin assembly and disassembly and regulated by the flow speed of actin. Our experiments further suggest that TCRs are structurally linked to the actin cytoskeleton through the Arp2/3 complex. On the other hand, we found that myosin II motor activity was dispensable for maintenance of traction stresses, but was important for traction stress generation. Finally, we investigated calcium influx in Jurkat T cells when activated on substrates of physiologically relevant stiffnesses. Our results highlight the importance of cytoskeletal forces for receptor activation in T cells.

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Persistent Activation of Signal Transduction Networks Induces a Novel Mechanism of Cell Death
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The directed migration of cells in response to chemical cues, known as chemotaxis, plays a critical role in normal physiology and disease pathogenesis. Knowledge of the molecular mechanism of chemotaxis is critical to our understanding of the process of metastasis and for developing new therapies to prevent it.
During chemotaxis 'front' proteins are recruited to extending pseudopods, which themselves are concentrated at the cell's anterior. Conversely, 'back' proteins dissociate from nascent pseudopods.
The Ras/TorC2 pathway has recently been shown to be important for chemotaxis in Dictyostelium. A similar conserved mTORC2 pathway has since been shown to play a role in neutrophil chemotaxis. The discovery of the importance of this pathway in chemotaxis challenges the conventional linear, PIP3-centric view of chemotaxis, in favor of a model involving a complex network of parallel pathways connected via feedback mechanisms.
In an attempt to decipher the mechanism of chemotaxis we generated pairwise mutations activating front protein RasC, and inactivating back-protein PTEN. Here we report that this combination of RasC and PTEN perturbations trapped cells in a globally activated or 'front-state'. Front-state ''clamped'' cells in Dictyostelium were extremely spread and flattened. Most surprising was the susceptibility of these altered cells to death by cytoplasmic fragmentation.
Front state clamped cells displayed global elevations of front protein signaling at the cortex. Levels of actin-binding probe GFP LimE at the cortex of front-state cells were elevated, as they were at the peak of the chemotactic response at the tips of pseudopods in control cells.
The promise of this research is rooted in our discovery of a potentially specific vulnerability of eukaryotic cells with a combination of mutations to PTEN and Ras; two genes commonly mutated in human cancers.

709-Pos Board B489
Acute Mechanical Stimulation Activates the Chemotactic Signaling Network
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Cells migrate in a directional manner in a variety of conditions, including chemoattractant gradients, electric fields, or under the influence of shear flow. Chemotaxis, or directed migration up a chemical gradient, is the best understood of these processes, and involves activation of multiple parallel signal transduction pathways that transmit the input from the chemoattractant receptor to the cytoskeletal network, leading to biased pseudopod projection in the direction of the gradient. Many components of the chemotactic signaling network display a polarized distribution in migrating cells. Although some of these markers have also been observed in cells migrating in an electric field or under shear flow, it is unclear whether these processes involve activation of a similar signaling network, or how they trigger cell migration. We found that acute mechanical stimulation of Dictyostelium cells leads to phosphorylation and activation of multiple components of the chemotactic signaling network, including PKB and ERK. Furthermore, using a microfluidic device, we demonstrated that application of shear flow for two seconds triggers translocation of leading and lagging edge markers to and from the cortex, respectively, similarly to global stimulation with a chemoattractant. Remarkably, the signaling network activated by acute mechanical stimulation displayed many behaviors characteristic of the chemotactic signaling network, including a refractory period, which is indicative of the system's excitatory nature. Simultaneous inhibition of multiple signaling pathways, including PI3K, PLA2 and TORC2, did not block the response to acute mechanical stimulation. In contrast, the response depended on the presence of an intact actin cytoskeleton, as well as Ca^{2+} flux mediated by the IP3 receptor homolog. Overall, these findings provide insights into the mechanism of shear stress induced migration, as well as a novel approach for studying the properties of the chemotactic signaling network without input from a chemoattractant.

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Pharmacological Activation of Myosin II to Correct Pancreatic Cancer Cell Mechanics
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Pancreatic ductal adenocarcinoma (PDAC) annually affects 44,000 people in the U.S. and has an abysmal five-year survival rate of around 6%, which is nearly unchanged over the past 40 years. Pharmacological strategies for treating cancer have primarily focused on inhibiting cell growth through specific genetic pathways, which typically either fail to abolish the disease or lead to compensatory regulatory changes and subsequently, to drug resistance. Importantly, alterations in mechanical properties are a common feature of cancer cells, yet targeting cell mechanics remains an under-utilized approach for drug development.
Here we develop a system for targeting cell mechanics for the discovery of novel therapeutics. We designed a live-cell, high-throughput chemical screen to identify mechanical modulators in *Dictyostelium discoideum.* We characterized 4-hydroxyacetophenone (4-HAP), which increases the cellular cortical tension by enhancing the cortical localization of the mechanoenzyme myosin II, independent of myosin heavy-chain phosphorylation regulation. To shift cell mechanics, 4-HAP requires myosin II, including its full power stroke. We further establish that changes in key cytoskeletal protein distributions correlate with the changes in the biomechanical profile of PDAC progression. In addition to actin-crosslinkers, we detect that non-muscle myosin II distributions vary across PDAC states: specifically myosin IIA increases, myosin IIB decreases, and myosin IIC increases in metastatic cells. We further demonstrate that invasive pancreatic cancer cells are more deformable than normal

pancreatic ductal epithelial cells, a mechanical profile that was partially corrected with 4-HAP. Tests of 4-HAP in mouse models of metastatic pancreatic disease are underway. Overall, 4-HAP modifies nonmuscle myosin II-based cell mechanics across phyla and disease states and provides proof-of-concept that cell mechanics offer a rich drug target space, allowing for possible corrective modulation of tumor cell behavior.

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SH3 Domain of C-Src Regulates its Dynamic Behavior in the Cell Membrane

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Src family kinases are major non-receptor tyrosine kinase in cells and Src-mediated signal transduction involves various cellular functions. In activation process, Src molecules translocate to cell membrane and the subdomains in Src, SH2 and SH3 domains, are exposed. For Src to serve as a kinase, the interaction with its substrates via SH2 and/or SH3 domains is required. Although the activation mechanism of Src has been well-studied, the dynamics of Src at the cell membrane is still unclear. In this study, we examined the role of Src subdomains, especially SH2 and SH3 domains, on the dynamics of Src at the cell membrane. To achieve this, we constructed PAmCherry-tagged wild-type Src (SrcWT), SrcW121A and SrcR178A mutants that decrease the binding of Src to its substrate(s) via SH3 and SH2 domains, respectively, and traced individual Src molecules in the cell membrane with gentle activation of PAmCherry. SrcWT dynamically moved on the cell membrane in the range of 0.27 ± 0.01 μm2/s within a few seconds. The dynamics of SrcR178A mutant was comparable with that of SrcWT, whereas SrcW121A mutant exhibited less mobility (0.16 ± 0.01 μm2/s) at the cell membrane compared with SrcWT. Since both SrcW121A and SrcR178A mutants showed higher phosphorylation level than SrcWT, the result indicates that the less mobility of SrcW121A in the cell membrane seems not to depend upon Src activation status. We further demonstrate that SrcW121A mutant showed ~30% increase in the Src molecules residence time at focal adhesion compared with SrcWT, which is mediated by slower dissociation from adhesion site. Taken together with enhanced localization of SrcW121A at focal adhesion, our findings show that the SH3 domain of Src molecules governs dynamics of Src at the cell membrane, which may be involved in the rapid signal transduction in cells.

712-Pos Board B492

Insights on RGD-Based Peptide Interactions with Integrin Receptors from Atomistic Simulations

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Here we develop a method for generating atomistically derived input parameters for a multiscale methodology to predict surface adhesion for functionalized nanocarriers. This methodology involves two primary steps: (i) AutoDock for determining the ligand-binding pocket and equilibrium-binding energy for receptor-peptide interaction, and (ii) an atomistic PMF calculation methodology for deriving the force vs. distance curves which serves as an input for the multiscale model. We also share some fundamental understanding of the differences in binding behavior due to changes in peptide sequences, especially the residues flanking the key binding mediator: the RGD. These differences in behavior at the binding pocket can be leveraged to better design the functional peptides to enable desired binding of cells to surfaces.

713-Pos Board B493

h2-calponin Gene Knockout Increases Traction Force of Mouse Fibroblasts in vitro

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Cell traction force (CTF) plays a critical role in controlling cell shape, enabling cell motility, and maintaining cellular homeostasis during various biological processes such as wound healing, angiogenesis, and cancer metastasis. It has been demonstrated that h2-calponin, an actin binding protein found in smooth muscle and non-muscle cells including epithelial cells, endothelial cells, macrophages and fibroblasts, plays a role in regulating the actin cytoskeleton activities in cell adhesion, migration and cytokinesis. We recently found that knockout (KO) of h2-calponin gene increased cell motility when compared to wild-type (WT) cells. This finding indicated a potential involvement of h2-calponin in producing CTF. The present study investigated the role of h2-calponin in mouse fibroblast traction force. Primary fibroblasts were isolated from leg muscles of h2-calponin KO and WT mice and analyzed using CTF-microscopy. CTF-microscopy is the current state-of-the-art method to determine CTF in a cell spread on a two-dimensional substrate. Using CTF-microscopy, we determined the root-mean square traction force, the total strain energy, net contractile movement produced by mouse fibroblasts cultured on a thin layer of 8-kPa polyacrylamide gel containing fluorescent beads of 0.2 μm in diameter. The results showed that h2-calponin KO fibroblasts had greater traction force than WT control. In comparison to WT cells expressing abundant tropomyosin-2, h2-calponin KO fibroblasts lost tropomyosin-2, a phenotype mimicking that of metastatic cancer cells. H2-calponin KO fibroblasts also adhered to cultural substrate slower than WT control, had smaller cell spreading area, and rounded up faster during trypsin treatment, supporting the role of h2-calponin in stabilizing the actin cytoskeleton. Our findings indicate that h2-calponin has an inhibitory role in the production of CTF, consistent with the increased motility of h2-calponin-null cells. Further studies on the mechanisms of h2-calponin-mediated CTF regulation and cell motility are underway.

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Deletion of h2-calponin in Macrophages Facilitates Cell Motility and Lipid Clearance: A Novel Mechanism to Attenuate Arterial Atherosclerosis

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Arterial atherosclerosis, a major cardiovascular risk condition, is a chronic inflammatory disease. The atherosclerotic plaques are built up by excessive lipid deposition and accumulation of apoptotic immune cells. Macrophages migrate into atherosclerotic lesions and function in scavenging extracellular lipid and mediating the inflammatory process. Lipid-laden macrophages transform into foam cells and loss their migration ability. The retention of foam cells at arterial intima promotes the growth of atherosclerotic plaques. Calponin is an actin filament-associated protein and its h2 isoform regulates cell proliferation, migration and other cell motility-based functions. We previously demonstrated that removal of h2-calponin in macrophages enhances cell migration and phagocytosis. Deletion of h2-calponin in macrophages significantly attenuated the development of inflammatory arthritis in mouse models (our unpublished results). In the present study, we investigated the function of h2-calponin-null mouse macrophages and foam cells in lipid clearance as well as their migration and transendothelial migration abilities. Foam cells are produced in culture by loading mouse peritoneal macrophages with acetylated low density lipoprotein. Lipid phagocytosis was quantified using Oil Red O staining of intracellular lipid droplets. Migration and transendothelial migration were examined using Transwell assay system. Foam cell apoptosis was studied using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The results showed that h2-calponin-null macrophages exhibit significantly higher lipid engulfment and faster migration and transendothelial migration than wild type controls. The h2-calponin-null foam cells retained higher migration capacity than that of wild type cells, which potentially facilitates migrating out of the arterial intima, reducing accumulation of apoptotic cells, and attenuating atherosclerotic lesions. The data demonstrate that h2-calponin is a novel molecular target for modulating macrophage functions and the development of new therapeutic approaches to the prevention and treatment of atherosclerosis.

Membrane Pumps, Transporters, and Exchangers I

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Arrayed Lipid Membranes on Femtoliter Chambers Allow Highly Sensitive Detection of Ion Translocation Catalyzed by Transporter Protein

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Femtoliter reaction chamber array enables highly sensitive and quantitative biological assays such as single-molecule enzymatic assay, and digital PCR. Although femtoliter chamber arrays are very powerful for protein science as well as for biomedical applications, most of them have been in general limited to use for water-soluble proteins, due to the technical difficulties in preparing uniform and stable lipid bilayers. Here, we report an arrayed lipid bilayer chamber system (ALBiC) that displays a sub-million of femtoliter chambers, each equipped with micron-size electrodes, and sealed with a stable lipid