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 were 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.

711-Pos  Board B491
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 μm/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 μm/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 Atomic 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 atomic 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, cell migration, and cell proliferation. Recently, we 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 lose 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

715-Pos  Board B495
Arrayed Lipid Membranes on Femtoliter Chambers Allow Highly Sensitive Detection of Ion Translocation Catalyzed by Transporter Protein
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Arrayed lipid reaction chamber array enables highly sensitive and quantitative biological assays such as single-molecule enzymatic assay, and digital PCR. 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 reaction chamber array (ALBC) that displays a sub-million of femtoliter chambers, each equipped with micron-size electrodes, and sealed with a stable lipid