mouse sperm by a cAMP/PKA-mediated pathway with a pharmacological profile suggestive of a Slc29a (ENT) equilibrative nucleoside transporter. Using qRT-PCR we find in adult mouse testis 160- and 32-fold greater expression of Slc29a1 than of the other surface-membrane ENT transporters Slc29a4 and Slc29a2. However, Slc29a1 protein was not found on mature sperm using immunocytochemistry. Moreover, wildtype and Slc29a1-null mice accelerate at similar rates (1.5-2.0 Hz min^{-1}) in response to Cl-dAdo indicating that Slc29a1 is not required for Cl-dAdo action. Consistent with this observation, the accelerating action of Cl-dAdo resists the Slc29a-selective inhibitor nitrobenzylthioinosine (NBTI; 10 μM). The accelerating action of Cl-dAdo additionally resists replacement of external Na^+ with $NMDG^+$ indicating that Slc28a concentrative nucleoside transporters (CNTs) also are not required. Interestingly, the Adenosine A3 receptor-selective agonist Cl-IB-MECA (25 μM) is nearly as effective as Cl-dAdo in accelerating sperm beat frequency, suggesting a possible role for cell surface A3 receptors in Cl-dAdo-mediated increases in sperm motility. Two A3 isoforms are expressed in the mouse Adora3i1 and Adora3i2; Adora3i2 expression is testis specific. Adora3i1 null sperm increase beat frequency in response to both Cl-IB-MECA (25 μM) and Cl-dAdo (25 μM), so this isoform is not needed for sperm response to adenosine. Sperm response to Cl-dAdo and Cl-IB-MECA is diminished after pertussis toxin treatment of cells suggesting the receptor is $G_{\alpha i/o}$ coupled. We are currently testing functionality of the testis-specific novel Adora3i2 isoform in a heterologous system. *Support from U54-HD12629 of the SCCPRR program of NICHD. L.A.B. supported in part by 5-T32-HD007453.*

2576-Pos

Novel Receptor-Mediated Endothelial Cell Chemotaxis

Amir Shamloo, Frank Kuhnert, Vir Choksi, Calvin Kuo, Sarah Heilshorn.
Stanford University, Stanford, CA, USA.

The directed migration of endothelial cells away from existing blood vessels is a critical step during cancer progression. Specific receptor-ligand interactions initiate intracellular signal transduction pathways and asymmetric cytoskeletal reorganization, leading to migration towards ligand sources. Disruption of these receptor-ligand interactions is a common goal in cancer drug development, and a key hurdle is the discovery of new receptor-ligand partners that are suitable targets. The G-protein-coupled receptor 124 (GPR124) is enriched in the endothelium of the developing brain and regulates embryonic CNS angiogenesis; however, its ligand and its potential role in endothelial cell chemotaxis are unknown. We examined the migration of mouse brain-derived endothelial cells (bEnd3) within custom microfluidic devices capable of generating stable concentration gradients of chemotactic ligands. The bEnd3 were genetically modified either to overexpress (GPR124+) or knock down (GPR124-) receptor expression. GPR124+ cells were observed to chemotax in response to a 0.025% gradient/micron of embryonic brain cortical cell conditioned medium (CM), while GPR124- cells followed random walk statistics in identical gradients. Mathematical analysis of the cell migration pathways (n~100 for each condition) revealed that net migration displacement, migration persistence time, and migration speed were increased for GPR124+ cells over GPR124- cells in both CM gradients as well as uniform CM concentrations. This suggests that in addition to being able to initiate asymmetric cytoskeletal reorganization, GPR124 signaling also triggers a general enhancement in cell motility. GPR124+ cells were also found to migrate towards VEGF-depleted CM, demonstrating that GPR124-mediated chemotaxis is independent from VEGF-mediated chemotaxis, a widely studied ligand of endothelial cells and a common cancer drug target. GPR124+ cells did not chemotax towards CM of 193T cells, a negative control kidney cell line. These results demonstrate utility of a new quantitative microfluidic platform to identify novel receptor-ligand partners for potential cancer drug development.

2577-Pos

Mobility of G Proteins is Heterogeneous and Polarized During Chemotaxis

Freek van Hemert, Milena Lazova, B. Ewa Snaar-Jagalska,
Thomas Schmidt.
Leiden University, Leiden, Netherlands.

The interaction of G-protein-coupled receptors with G proteins is a key event in transmembrane signal transduction leading to vital decision-taking of the cell. Here we applied single-molecule epifluorescence microscopy to study the mobility of both the Gβγ and the Gα2 subunits of the G protein heterotrimer in comparison to the cAMP-receptor responsible for chemotactic signaling in Dictyostelium discoideum. Our experimental results suggest that ~30% of the G protein heterotrimers exist in receptor pre-coupled complexes. Upon stimulation in a chemotactic gradient this complex dissociates, subsequently leading to a linear diffusion/collision amplification of the external signal. The further observation of partial immobilization and confinement of Gβγ in an agonist, F-actin and Gα2-dependent fashion led to the hypothesis of functional nanometric domains in the plasma membrane that locally restrict the activation signal and in turn lead to faithful and efficient chemotactic signaling.

2578-Pos

Physical Properties of Fibrinogen Substrates Control Integrin Mediated Cell Adhesion

Ivan Yermolenko[1], Alexander Fuhrmann[2], Tatiana Ugarova[1], **Robert Ros[2]**.
[1]Center for Metabolic Biology, School of Life Sciences, Arizona State University, Tempe, AZ, USA, [2]Center for Biological Physics, Department of Physics, Arizona State University, Tempe, AZ, USA.

The physical properties of substrates are known to control cell adhesion via integrin-mediated signaling. Recently, we have shown that binding of fibrinogen to the surface of fibrin gel prevents integrin $\alpha_M\beta_2$-mediated leukocyte adhesion by creating an anti-adhesive layer. Furthermore, fibrinogen immobilized on various surfaces at high density supports weak cell adhesion whereas at low density it is highly adhesive.

To gain an understanding of the mechanism underlying differential cell adhesion, we extended the cell adhesion assays to platelets carrying integrin $\alpha_{IIb}\beta_3$. The results showed a similar behavior indicating that the process is independent of the type of integrins. In order to quantify the adhesion forces, we applied single cell force spectroscopy (SCFS). In this assay, a single cell is attached to a tipless cantilever of an atomic force microscope (AFM) and force-distance curves for different surfaces are acquired. For cells carrying $\alpha_M\beta_2$-integrins we found significant lower adhesion forces for high- compared to low-density fibrinogen substrates.

Furthermore, we analyzed the adhesive behavior of fibrinogen surfaces using force spectroscopy with a silicon nitride AFM tip. These experiments, unrelated to the cells and integrins, show similar behaviors as the cell adhesion assays. AFM images of the different substrates indicate that fibrinogen deposition at high density results in an aggregated multilayered material characterized by low adhesion forces. However, low-density fibrinogen produces a single layer in which molecules are directly attached to the solid surface resulting in higher adhesion forces.

The data suggest that deposition of a multilayered fibrinogen matrix prevents stable cell adhesion by modifying the physical properties of surfaces resulting in reduced force generation with implications for hemostasis and biomaterial applications.

2579-Pos

Mechanochemical Signaling in Glomerular Podocytes

Anna H. Klemm[1], Sandra Kienle[1], Navid Bonakdar[1],
Wolfgang H. Goldmann[1], Jose-Luis Alonso[2].
[1]Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany, [2]Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA.

Kidney glomeruli function as filters, allowing the passage of small solutes and waste products into the urinary space, while retaining essential proteins and macromolecules in the blood stream. They achieve this through a filtration apparatus comprised of three layers, a fenestrated glomerular endothelium, a basement membrane (GBM) and an epithelial cell layer. Glomerular epithelial cells (podocytes) culminate into interdigitating foot processes (FPs) between adjacent cells. Specialized structures known as slit diaphragms (SDs) function as modified adherens junctions connecting podocyte foot processes. These structures are under constant mechanical stress due to fluid pressure, driving filtration across the barrier. Disruption of the SDs or podocyte damage can lead to progressive loss of protein into the urine (proteinuria) and ultimately to kidney failure. Dysregulation of the podocyte actin cytoskeleton has been implicated in mechanisms of proteinuria.

Podocyte adhesion on collagen activates the FAK/ERK pathway, a known mediator of F-actin assembly. Here, we mechanically stimulated adherent podocytes using a cell stretcher. A 20% strain at 1 Hz for 20 min increased ERK phosphorylation compared to control. We observed a peak after 2 min and thereafter an exponential decrease. This suggests that podocytes are responsive to early external strain, which might be transduced into a chemical signal capable of modulating the actin cytoskeleton. We hypothesize that adhesion via integrin receptors triggers mechanochemical signals which may in turn affect cell-cell and cell-GBM connections. Currently, we are studying the effects of cytoskeletal organization/regulation in wildtype and mutated podocytes, with a focus on determining cell mechanical properties using magnetic tweezers and atomic force microscopy.

2580-Pos

A Model of Atherosclerosis Plaque Formation and Development

Nicolas Meunier, Julien Dambrine, Jean-Gabriel Houot, Michel Boynard, Annie Raoult.
Universite Paris Descartes, Paris, France.

Atherosclerosis is a progressive disease characterized in particular by the accumulation of lipids and fibrous elements in arteries. Over the past decade,