and soluble factors, while a proteomic comparison underscores both differences and overlaps in differentiation pathways.

2842-Pos Board B272
Altered Contractile Machinery in Airway Epithelial Cells in Response to Cigarette Smoke
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Chronic obstructive pulmonary disease (COPD) is the 3rd leading cause of death in the US with cigarette smoke being the primary insult leading to disease progression. While the lung epithelium is the site that makes initial and primary contact with the inhaled cigarette smoke, we do not understand the role of cytoskeleton and cell mechanics in the airway response to cigarette smoke. We know that cytoskeletal proteins, such as myosin II, actin, alpha-actinin, the catenins, and E-cadherin, are involved in cell-cell adhesion formation and barrier function of the lung. Furthermore, E-cadherin levels decrease in primary epithelial cells from COPD patients. Therefore, we hypothesized that cigarette smoke drives key cytoskeletal protein changes that lead to alterations in barrier function and thereby influence the development of chronic lung disease. To test this, we studied major cytoskeletal (actin and nonmuscle myosin II isoforms) and cell adhesion proteins (E-cadherin) in normal human bronchial epithelial cells (NHBE, collected directly from human patients) or 16HBE cells (primary human bronchial epithelial cell line) grown on an air-liquid interface. Using a Vitrocell smoke chamber, we found that in response to acute smoke exposure (four cigarettes in 24 hrs), myosin IIB and actin assembled into apical stress fibers, which are not normally found in airway epithelial cells. Cigarette smoke exposure also resulted in decreased total E-cadherin levels as well as decreased E-cadherin at the basolateral surface. Thus, cigarette smoke promotes dramatic acute cytoskeletal changes that likely lead to altered cell mechanical properties (tension, elasticity), which in turn lead to reduced epithelial barrier function and our data suggest that epithelial barrier function is critical in dictating chronic tissue responses that contributes to COPD development and progression.

2843-Pos Board B273
Primary Cilia Length is Critical to Cellular Mechanotransduction
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Mechanotransduction is an essential cellular function in a variety of tissues including bone, kidney, and endothelia. The primary cilium is a single immotile organelle protruding from the surface of these cells, and has repeatedly been demonstrated as a critical mechanotransducer in these cell types. When the primary cilium is impaired, these cells have an abrogated response to mechanical stimulation. Here, we demonstrate a method to enhance cellular mechanotransduction, by increasing primary cilia length. To elongate primary cilia, we treated MLO-Y4 osteocytes with fenoldopam, lithium chloride, or vehicle control for 16 hours. We then subjected these cells to oscillatory fluid flow for 1 hour at 1 Hz and 1 Pa wall shear stress. Immediately following flow, cells were lysed, and mRNA expression was analyzed. Cells with longer cilia displayed increased expression of osteogenic markers cyclooxygenase-2 and osteopontin, compared to vehicle control, suggesting that these cells are more mechanosensitive. To discern the role of fenoldopam on cilia length from other cellular processes, we treated cells with IFT88 siRNA_IFT88 is critical for primary cilia formation_which resulted shorter cilia and an abrogated response to fluid flow. Fenoldopam was able to restore cilia length in cells with impaired cilia formation, and rescued flow-induced osteogenic signaling. Together, these data suggest that cells with longer cilia are more mechanosensitive, and that cellular mechanotransduction can easily be modulated by pharmacologically lengthening primary cilia. Primary cilia-mediated mechanotransduction is a critical function in an array of cell types, and numerous diseases, such as polycystic kidney disease and Bardet-Biedl Syndrome, are characterized by impaired cilia function. This work suggests a potential therapeutic strategy to combat such conditions.

2844-Pos Board B274
Structure of an Inner-Ear Protocadherin-15 Fragment with an Atypical Calcium-Free Linker
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Tip links are protein filaments essential for hearing and balance. They convey force to and gate inner ear hair cell transduction channels to mediate sensory perception. Cadherin-23 and protocadherin-15 form tip links through a calcium-dependent heterophilic interaction of their extracellular domains, which are comprised by multiple modules termed extracellular cadherin ''EC'' repeats. These EC repeats are similar but not identical to each other in terms of sequence and structure, often featuring highly-conserved calcium-binding sites at the linker region between them. Recent sequence analyses and structures of cadherins revealed unusual calcium-free inter-repeat linkers in some protocahderins and other non-classical cadherins. Bound calcium ions have been shown to provide structural rigidity to cadherins, thus the presence of unusual sites may confer higher flexibility and perhaps affect the tertiary and quaternary arrangement of cadherins that harbor them. Analysis of the protocadherin-15 sequence shows unusual calcium-binding sites in some of its inter-repeat linkers. Here we present the x-ray crystal structure of repeats EC8-10 refined at 3.3 Å resolution, which shows an EC9-10 calcium-free linker that alters the linear arrangement of protocadherin-15's EC repeats. We suggest that several unusual features of these repeats affect the overall elastic response of protocadherin-15 that is relevant for tip link function in sensory perception.

2845-Pos Board B275
Force-Free Transition from Closed to Open MscL: A Molecular Dynamics Study
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The mechanosensitive channel of large conductance (MscL) is exceptionally important in bacterial cells as when the cellular pressure becomes too high and the membrane tension rises, these channels rapidly open allowing an efflux of cellular contents that prevents the cell from bursting. As there are no homologues of this channel in humans, it is seen as a promising new target for antibiotics, something that is critically important given the increasing resistance to existing drugs. While MscL has been studied for many years, exactly how it senses membrane tension to go from a closed to an open state is still unknown, however, it has been observed that mutations in the gating region strongly affect the tension required to open the channel. We have applied molecular dynamics to study wild type MscL and three channel mutants, G22E, G22S and G22N-G26N that each have lower gating tension thresholds than wild type. In the absence of any external force we have observed specific and interesting differences in the propensity of each channel to transition towards the open state. On the timescale sampled for each mutant channel (0.5-2.25 μs), we have observed sub-conductant states of MscL allowing both the measurement of an appreciable current and atomistic detail of how the transition from a closed to a partially open channel occurs. This provides a model for how mechanical forces can be converted into a physiological response.

2846-Pos Board B276
Patch Clamp Characterisaton of the Effect of Cardiolipin on the Bacterial Mechanosensitive Channels of Small (MscS) and Large (MscL) Conductance
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The bacterial mechanosensitive channels MscS and MscL respond to membrane tension by opening when the bacterium experiences hypoosmotic shock conditions to prevent cell lysis [1]. Environmental factors such as cholesterol [2] and cations/anions [3] also affect the gating behaviour of these channels. We have previously shown that addition of the negatively charged lipid cardiolipin to POPE/POPC membranes cause rapid and flickery behaviour of MscS [4]. Here, in an expanded study to include MscL, we compare the gating kinetics and pressure sensitivitiy of the channels with and without cardiolipin in both azolectin and mixtures of pure lipids DOPE/DOPC. In azolectin liposomes, mixtures of 10% cardiolipin abolish hysteresis of MscS, but MscL remains largely unaffected, indicating it may stabilise the closed state of MscS. Compared to the azolectin, mixtures of DOPE/DOPC abolish the hysteresis gating of MscS even in the absence of cardiolipin and addition of cardiolipin increases the opening and closing thresholds of both MscS and MscL. These results suggest that cardiolipin shows a significant effect on the mechanosensitive gating of both MscS and MscL.
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Supported by a JSPS Fellowship to Y.N., APP1079398 grant from NHMRC to B.M. and Griffith University Project grant to A.B.