is assumed that also in non-erythrocytes the membrane skeleton is mostly composed of heterotetramers of α-II and β-II spectrin. Because of α-spectrins' potential role as mechanotransducers, we investigate the effect of mechanical stimulation on the spectrin-ankyrin B membrane skeleton of NIH 3T3 fibroblasts and murine C3H 10T½ cells. Using quantitative fluorescence microscopy and biochemical techniques, the absolute changes in cellular spectrin and ankyrin B content were determined. Before stimulation the spectrins and ankyrin B make up more than 10% of cytosolic and membrane proteins. Thus, they constitute a much more significant part of the non-erythroid cytoskeleton in these cells than previously assumed. Interestingly, both cell types contain all four major isoforms of spectrin. However, while α-II spectrin is the dominant α spectrin in 3T3, in 10T½ cells it is the 'erythroid' α-I spectrin. Also, at least half of the α spectrins do not have β-spectrin counterparts and thus cannot be part of the classical heterotetramere structure. Mechanical stimulation decreases the overall spectrin amount by up to 60% with α-spectrins exhibiting the largest loss. In contrast, the number of ankyrin B copies increases by almost 30%. Furthermore, the fraction of polyubiquitinated spectrin actually increases, which in part could account for the reduction in α-spectrins and is in line with their proposed mechanically enhanced self-ubiquitination activity.

701-Pos Board B481

Vimentin Intermediate Filament Mechanics in Cells under Shear Stress

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Cells experience a wide range of mechanical stimuli depending on their environment. Cellular responses to mechanical stress are thus an important aspect of cell mechanics to understand. For example, cytoskeletal rearrangement has been observed when shear stress is applied to cells. We are interested in the contribute of this rearrangement to changes in local material properties and mechanics. Using microrheological tools, we are able to better understand these changes in the larger picture of cell mechanics.

702-Pos Board B482

Electomechanical Model for Non-Excitable Cells

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Mechanical and electrical properties of cells are crucial in regulating a wide range of cellular processes, such as motility, cancer metastasis, and embryogenesis. Cell size and membrane potential are both highly regulated properties in eukaryotic, non-excitable cells. The proposed model explains the interplay between mechanical, electrical, and biochemical properties of a cell, and can have important implications for pathology and medicine. The combination of osmosis-driven water flow, passive ion flux, ion pumping, signaling, and cytoskeletal forces provide a mechanism for how cells control their volume and membrane potential. For example, the model predicts that cell volume increases during depolarization and decreases slightly during hyperpolarization. Whole cell voltage clamp experiments provide a technique for measuring cell volume while controlling membrane potential. Experiments done on different eukaryotic, non-excitable cancer cell lines validate the model.

703-Pos Board B483

The Role of Cellular Mechanical Properties in Microenvironment-Dependent Behavior

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Understanding the role of mechanics in cell behavior is necessary for effective development of tissue engineering therapies. This study observed how cellular mechanical phenotype influenced cell morphology and actin organization on substrates coated with different extracellular matrix ligands. Inherent cell stiffness, represented by Young's modulus, for MG-63 (osteosarcoma) and SH-SY5Y (neuroblastoma) cells was mechanically determined using atomic force microscopy. These cells have dissimilar moduli when probed over the nucleus (1.3 ± 0.5 and 0.26 ± 0.15 kPa for MG-63 and SH-SY5Y cells, respectively). To investigate the effect of substrate compliance, cells were cultured on polyacrylamide gels ranging from 0.3 to 12 kPa and functionalized with fibronectin, laminin, or collagen-1. We hypothesized that cells would spread and attach more successfully to substrates with an elastic modulus equal to or greater than that of the cell, provided physiologically relevant ligands are present. Cellular responses were captured daily over 4 days using fluorescent microscopy. MG-63 cells showed two different organizational phenotypes: a multi-cell, spheroid-like structure and a spread monolayer. The threshold substrate compliance required for MG-63 cells to organize into either phenotype was designated by the cells' own stiffness, most clearly observed on collagen-1 coated gels. While SH-SY5Y cells exhibited similar phenotypic organizations when plated on varying substrates, no mechanical compliance threshold was observed. However, SH-SY5Y cells exhibited greater tendencies toward forming spheroids when plated on fibronectin. These morphological changes show a possible correlation between the cells' characteristic mechanical phenotypes and their relationship to the microenvironment. Cells exhibiting less compliant phenotypes may have more substrate stiffness-dependent behavior, whereas more compliant cell types may have more ligand-dependent behavior. These findings indicate that cellular mechanosensing is influenced not only by the mechanical and biological characteristics of the microenvironment but also by the inherent mechanical phenotype of cells comprising a population.

704-Pos Board B484

Characterizing Mechanical Forces during B Cell Responses

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B cells are components of the adaptive immune response that produce high-affinity antibodies that neutralize and provide immunity against infections. Their responses are initiated when the B cell receptor (BCR) binds foreign antigen, triggering intracellular signaling that leads to B cell activation. Current evidence suggests that to elicit a full immune response involving development into high-affinity memory and plasma cells, B cells need to acquire the antigen from immune synapses with antigen presenting cells, and then internalize, process and present it to receive T cell help. Recent studies from our group suggest that B cells use contractile force to physically extract antigen from the presenting membrane prior to endocytosis. In addition, it was shown that pulling on the BCR-antigen bond results in discrimination between high- and low-affinity interactions. This suggests that B cells mechanically test the strength of BCR-antigen bonds to actively regulate affinity discrimination, a process that is important for the efficient development of high-affinity antibodies. To characterize force generation in the B cell synapse, we combine live-cell imaging with molecular force sensors. For the first time, we resolve the spatiotemporal dynamics and magnitude of mechanical forces in the B cell synapse, giving us insight into how B cells use mechanical forces to actively regulate their responses to antigen binding.

705-Pos Board B485

Atomic Force Microscopy of Triple Negative Breast Cancer Cells: A Predictive Value of Mechanical Phenotype

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We applied Quantitative Nanomechanical Atomic Force Microscopy (QNM-AFM) to test a response of physical phenotype of triple negative breast cancer cells to treatment with proteasome inhibitors. Genetic screens identified TNBCs as addicted to the activity of the ubiquitin-proteasome pathway, the major intracellular venue of regulated protein degradation, served by the essential protease, the proteasome. We investigated two types of proteasome inhibitors: the drug bortezomib (Velcade®) targeting active sites of the enzyme and a novel allosteric inhibitor B1 interfering with stability of the 26S proteasome. Mechanical phenotype constitutes a sensitive indicator of physiological status of cells. The most explored mechanical parameters are cell elasticity (''softness'') and surface adhesiveness (''stickiness''). It has been established that cancerous transformation makes cells softer and less sticky. These differences are attributed to remodeling of the cytoskeleton and altered expression of membrane proteins. Therefore, the physical phenotype should have a predictive value as the early indicator of the cells' response to a drug treatment and disease stage. Indeed, it has been shown that exposure of cancer cells to anticancer drugs at least partially reverses their mechanical phenotype resembling healthy cells. Here we found that subjecting breast cancer cells to low doses of bortezomib increased their stiffness and adhesiveness about two times. Moreover, similar treatment with low doses of B1 induced almost a threefold increase of stiffness and a twofold increase in cell adhesion. Surprisingly, much higher doses of the drugs were required to inflict detectable changes in cells viability or morphology. The results point at the extraordinary sensitivity of the mechanical phenotype to detect cells' response to anticancer drugs. We are exploring the potential predictive value of AFM-based cell surface studies in a search for effective drugs or drug combinations.