quantify lamin stoichiometries in human hematopoietic stem cells and progenitors through different mature blood lineages. This approach reveals the hematopoietic lineage map of lamins, showing that lamin A varies by 4-fold, while the normally 'constitutive' lamin B varies by 30-fold. During differentiation, lymphoid and myeloid lineages show decreased total lamin intensity and pliable nuclei as measured by micropipette aspiration, consistent with their ability to transmigrate into circulation. In contrast, megakaryocytes (MKs) remain in marrow because their polyploid nuclei are too large and rigid, as indicated by high lamin levels; this nuclear anchorage allows MKs to extend membrane projections into blood, where shear generates circulating platelets. Erythroid lineages share the same progenitor with MKs and migrate into blood as enucleated RBCs, because of high lamin A intensity relative to B in the progenitors and stiff chromatin. Functional studies indicate that lamin A overexpression increases MK and erythroid differentiation by 2-fold, while the knockdown increases migration through pores by 2-fold. Interestingly, increasing the lamin A to B ratio by lamin B1 knockdown decreases nuclear deformability by up to 50%. Together, the study suggests that nuclear deformability is hierarchically programmed by differential expression of lamins during hematopoietic differentiation, which in turn influence the ability of cells to migrate through marrow.

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How Deeply Cells Feel: Nuclear Phenotypes Defined by Cellular Tactile Sensing

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Lacking eyes to see and ears to hear, cells physically sense their microenvironment and feel into the depths of a matrix by actively deforming their surroundings. To study how deeply cells feel, mesenchymal stem cells, as prototypical but particularly sensitive adhesive cells, were cultured on collagen-coated gels-based microfilms of controlled elasticity (E) and thickness (h). Cellular morphologies and nuclear deformations were distinctively smaller on soft compared with soft but thin or stiff films. As indicated by the transition from small to large spreading, the tactile length scale for mechanosensitivty was 6-10 microns. A small set of highly mechano-malleable nuclear envelope genes were identified by transcriptional profiling across tissues and in a dish. The transition between 'tense' and 'soft' nuclear phenotypes was delineated by Lamin-A,C overexpression and knockdown assays and involved inside-out modulation of nuclear envelope components, cytoskeleton, adhesions and extracellular matrix proteins. Inter-regulatory correlations between lamin-A,C and non-muscle myosin constitute a feedback between nucleus mechanics and cytoskeletal tension. Fluorescence recovery rates after photobleaching of a mini-library of phospho-mimetic Lamin-A,C mutants provided insights to early dynamics of nuclear remodeling upon matrix engagement. Taken together, our findings are indicative of nuclear phenotypes directed by cellular tactile sensing which links the regulation of gene expression with matrix physics.

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Regulation of Nuclear Shape and Function with Cell Elongation

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Many cellular events require dynamic changes in the shape and structural organization of the cell morphology and presumably affect gene expression by changing the nuclear shape. In this work, we investigate the effect of cell shape on the nucleus and the mechanism by which intracellular forces are transmitted to the nucleus. We analyzed the intracellular reorganization of individual endothelial cells plated on micropatterned substrates, imposing cells to spread on various aspect ratios. Specific drugs were used to alter each component of the cytoskeleton and we observed the spatial reorganization of the actin network, microtubules, intermediate filaments and focal adhesions, as well as the nuclear shape. Our data demonstrate the key role of the actin cytoskeleton in the adaptation of nuclear shape with cell elongation. Indeed, we show that the nucleus is subjected to pincer forces generated within the cytoskeleton via actin stress fibers. These intracellular forces drastically affect the nuclear shape and decrease the nuclear volume by 40-50% before attaining a state that is highly resistant to further deformation. Based on the quantification of cell traction forces by traction force microscopy, we propose a mechanical model that accounts for our observation and quantitatively predicts the nuclear shape. Our work also demonstrates that nucleus adaptation to cell elongation leads to a modification of nuclear functions. Indeed, DNA staining reveals an increase in chromatin condensation in highly deformed and compressed nucleus. We show that nuclear deformation in response of cellular elongation results in a strong decrease of cell ability to enter S phase and thus to proliferate. In conclusion, our results demonstrate that the shape of the cell is transposed by the actin cytoskeleton to the nucleus and suggest that it can alter the accessibility of genes to the transcription machinery.

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Stem Cell Nucleus Morphology is Modulated by Matrix Mechanics via the Cytoskeleton

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It is now widely accepted that cells physically interact with their surrounding in multiple ways and that these mechanical cues can be as important as biochemical ones. Especially striking was the demonstration that human mesenchymal stem cells (hMSCs) are mechano-sensitive and were shown to differentiate towards distinct lineages based on the stiffness of their environment. While this mechanically guided differentiation of hMSCs takes several days or weeks we focused on the early time interactions between cells and elastic substrates. Analyzing cell morphology and cytoskeletal structure of cells cultured on hydrogels of different Young's moduli we found that the total amount of contractile non muscle myosin IIa fibers increases monotonically with substrate stiffness similar to the trend in projected cell area. The aspect ratio of the cell and the order parameter of the acto-myosin fibers show a maximum on 11 kPa substrates, a rigidity comparable to the cell [1]. Hence, the organization of the acto-myosin cytoskeleton can be used as an early morphology marker for subsequent lineage specification. We then used novel hyaluronic acid based hydrogels to investigate the impact of a 3D environment and could show that matrix stiffness also dictates cytoskeleton organization in the third dimension [2]. Since stem cell differentiation happens in the nucleus, we analyzed the nuclear morphology of hMSCs in 2D and 3D environments and could demonstrate a strong coupling to the matrix elasticity that is mediated via acto-myosin stress fibers indicating a direct mechanical pathway from the extra-cellular matrix to the nucleus.

References:
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Lamin-A Levels Limit 3D-Migration but Protect against Migration-Induced Apoptosis

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Cell migration through dense matrix or tissue has often been seen to involve large nuclear contortions. The nucleoskeletal protein Lamin-A is variably expressed between cells and shown here to have a strong effect on 3D-migration of diverse cell types involved in disease or regeneration. Crawling through tissue is modeled by motility through micro-pores, with small decreases in Lamin-A producing large increases in net migration. Surprisingly, the sensitivity to Lamin-A changes is greatest when Lamin-A is low relative to constitutive Lamin-B's. Nuclear shape changes after micro-pore migration as well as nuclear response times in micropipette aspiration scale strongly with Lamin-A:B stoichiometry across cell types, revealing Lamin-A's role in nuclear plasticity and Lamin-B's role in nuclear elasticity. Lamin-A also protects against apoptosis induced by micro-pore migration, with deeply deficient cells showing defects in stress-resistance. Xenografts provide *in vivo* insight and show moderately low Lamin-A levels promote growth of the graft. The nuclear lamina thus acts as a physical impediment to motility and also promotes survival in withstanding the mechanical stresses of migration.

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Quercetin Exerts Deleterious Effects on the Reproductive Potential of Male Mice

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Oxidative stress is a leading cause of male infertility. To combat this, germ cells and spermatozoa are endowed with various enzymes, vitamins and proteins. Certain other components of food, including bioflavonoids also provide protection against free radicals. Present study analyzed the effect of quercetin, a bioflavonoid, on male reproductive function in adult mouse, after intra-peritoneal treatment with varying concentrations of quercetin (2, 8 and 20mg/kg b.wt.) for two weeks. Quercetin increased the generation of reactive