CP3: Nuclear mechanics in laminopathies: Jan Lammerding, Cornell University
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Associated With: TRD2, 3

Significance: Lammerding laboratory is a leader in studies on mechanotransduction – a process by which cells sense mechanical forces and deformations, and respond through cytoskeletal reorganization, biochemical signaling, and specific biological functions. His research specifically focuses on delineating the complex interplay between nuclear envelope and surrounding cytoskeleton. Lammerding Lab has demonstrated that mutations in the *LMNA* gene encoding the Lamin A/C proteins lead to impaired nuclear mechanics (see Fig. 1), which is responsible for defective mechanotransduction (1-6) and hence for the on start of a number of human genetic diseases including muscular dystrophies, cardiomyopathies, lipodystrophies and progerioid phenotypes (7-9). Beside laminopathies, nuclear mechanics is also altered in cancer cells (10) and plays an important role during cancer metastasis (11-13). It is vital to study nuclear mechanics and that of Lamin A/C deficient cells, in particular, to clearly understand the behavior and function of normal and diseased cells.

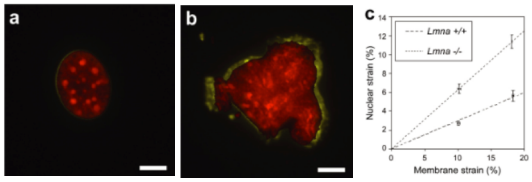

Fig. 1: (a) Wide-type and (b) lamin A/C deficient nuclei under similar substrate strain.(c) The lamin-deficient nucleus exhibits significantly greater strain (3).

Rheological measurements generally involve applying precisely controlled stresses and monitoring the resultant strains. When measuring nuclear mechanics, one challenge with the most common approaches such as micropipette aspiration, AFM indentation, and optical / magnetic tweezers (14) is that the mechanical loading / stimulation can often be only indirect since the nucleus is surrounded by cell cytoskeleton. One possibility is to probe isolated nuclei; however, due to the difficulty of matching the physiological buffer conditions of the nuclear interior and the lack of cytoskeletal tension, the morphology and hence mechanical properties of isolated nuclei are typically quite distinct from those within intact cells (15-17). Lammerding Lab also focuses on developing novel assays for nuclear mechanics. Specific examples include cellular strain assay in which cells are cultured on fibronectin-coated silicone membranes and subjected to well-defined uniaxial or biaxial strain, and microneedle manipulation assay that applies precisely controlled cytoskeletal strain at a defined distance from the nucleus while simultaneously imaging induced nuclear and cytoskeletal deformations (18-22). Despite these efforts, the exact mechanisms underlying laminopathies are not well understood due technology limitations.

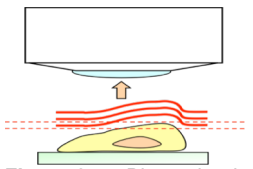
Fig. 2: Biomechanical assay based on 3-D resolved interferometric measurement of nanometer scale nuclear membrane fluctuations (TRD2.1)

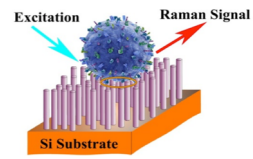

Fig. 3: Biomechanical assay based on nanopillar array (TRD 3.1).

Approach: LBRC has successfully applied transmission-type quantitative phase microscopy based assays (23, 24) for contact free measurement of biomechanical properties in red blood cells (24-30). Developing 3-D resolved interferometric biomechanical assays to study biomechanical properties of complex eukaryotic cells including their nuclei has been a major goal of LBRC in this cycle. Although we made major progresses in instrument development, we were not successful in developing a method with sufficient depth and temporal resolution and sensitivity resulting in progress on this CP. We now have the requisite technology at hand; the proposed design (TRD2.1) is based on multi-spot scanning confocal reflection phase microscope and promises necessary features such as 1 μm depth-sectioning, fast (0.1-1kHz) frame rate, and high (< 1nm axial motion) measurement sensitivity. In addition, LBRC will develop a complementary approach based on plasmonic nanoparticle-coated vertical nanopillar array with single-molecule detection sensitivity and surface selectivity (TRD 3.1). The proposed technology will enable non-invasive subcellular perturbations and simultaneous nanoscale monitoring of events at the cell surface. In addition to subjecting adherent cells to adjustable mechanical stimuli, the plasmonic nanoparticle nanopillar array will also feature simultaneous ultrasensitive detection of membrane biomarkers responsible for cell signaling, migration, and proliferation.

Push-Pull Relationship: To understand nuclear mechanics in Lamin A/C deficient cells, LBRC **pushes** for the development of highly sensitive non-invasive interferometric 3D biomechanical assays with depth-sectioning capability based on common-path multi-point scanning confocal reflectance phase microscope. We further **push** a complementary approach based on nanopillar array will also be developed to quantify sub-cellular responses to controlled mechanical stimuli. Successful studies of nuclear biomechanics in laminopathies is expected to further **pull** for integration of proposed 3D biomechanical assay with select fluorescence spectroscopic tools to simultaneously study biochemical changes such as DNA chromosomal damage.

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