UNDERSTANDING LINC COMPLEX PERTURBATIONS ON CARDIOMYOCYTE FORCE TRANSMISSION AND GENE EXPRESSION

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Introduction: Cardiomyopathy is a cardiac muscle-related condition that is the most common cause of sudden-death in young people (30 years or less)[1] and has been associated with cardiomyocyte (CM) mutations in LINC complex proteins, weakening the nuclear envelope. ***Studies have shown that mechanical forces on the heart throughout life have an effect on the cytoskeletal-nuclear interactions of CMs.*** Deformations in the nucleus causes genome reorganization, increasing patient propensity to cardiomyopathy. However, disrupting force transmission via the LINC complex is found to compensate for weakened nuclear membrane, increasing lifespan.[2]

Methods: We have found that cells containing nesprin-1 protein in the nuclear membrane with a dominant negative KASH domain (DN-KASH) (affecting membrane/LINC complex) have decondensed peripheral chromatin relative to control cells. Confocal imaging with immunofluorescence will allow us to see disruption of the KASH domain with infection of cells with DN-KASH virus after 48 hours. and differences in h3k9me2 marker concentration.

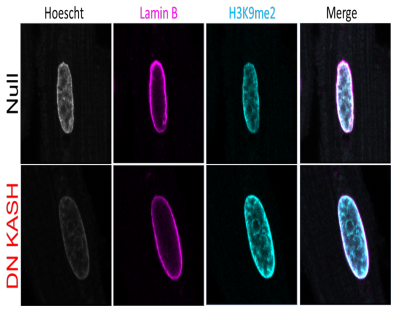

Figure 1: **IF against Lamin B and H3K9me2**

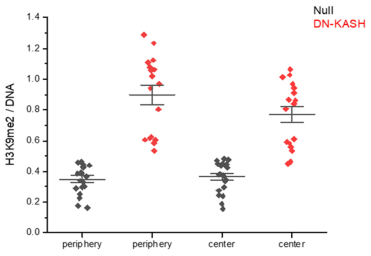

Figure 2: Quantitative H3K9me2 Concentration to DNA

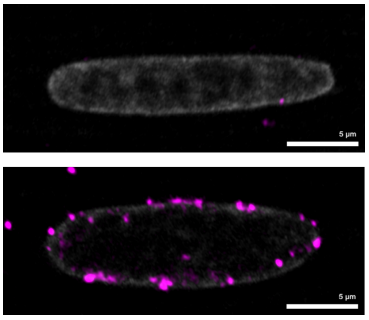

Figure 3: **PLA Null and DN-KASH**

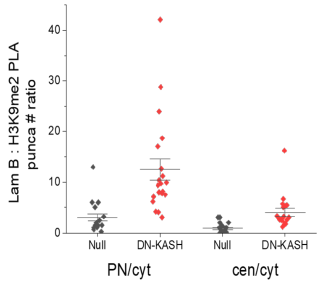

Figure 4: **Lam B : H3K9me2 ratio**

Results and Discussion: In null cells, lamin B is more concentrated at the nuclear periphery and H3K9me2 markers aren't as strong as opposed to DN-KASH. From figure 1, cells with a perturbed DN-KASH membrane have less lamin B due to increase in surface area, and higher signal of methylation, especially around the periphery. Using Proximity Ligation Assay (PLA) (figure 3), in DN-KASH cells, there's a significantly higher number of punctae around the nuclear periphery, suggesting interactions in disrupted LINC cells.

Conclusions: There is quantifiable evidence that forces exerted on the nucleus by the cytoskeleton are important in maintaining chromatin compaction, and thus the accessibility of DNA to transcription factors due to hyperacetylation or hypomethylation around histones.

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[1] Centers for Disease Control and Prevention

[2] Disrupting the LINC complex by AAV mediated gene transduction prevents progression of Lamin induced cardiomyopathy- Ruth Jinfen Chai et al