Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation

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1) Lamin-A and collagen levels scale with tissue stiffness

Tissues are soft like fat, which bears little stress, or stiff like bone which sustains high stress, but any systematic relationship to specific proteins in tissues or to general processes of differentiation is largely unknown. Proteomics analyses of tissues reveals the nucleoskeletal protein lamin-A follows polymer physics scaling as a function of tissue elasticity. Evidence suggests a mechano-sensitive regulation of the lamina, but the process of mechanical stimulation of molecular processes is poorly understood. Cysteine-shotgun mass spectrometry (CS-MS), a method capable of mapping the exposure of cysteine residues as proteins are stressed in complex biological systems, allowed us to identify stress-sensitive proteins. We find that lamin-A contains stress-sensitive domains with changes in protein conformation coupled to phosphorylation. Conformational changes are linked directly to transcriptional changes that cause disease in stiff tissue, but lamin-A levels in cultured cells also adjust to matrix elasticity. Differentiation of stem cells to fat or bone is respectively enhanced by low or high lamin-A levels, and that cause disease in stiff tissue, but lamin-A levels in cultured cells also adjust to matrix elasticity. Nuclear lamin-A scales with tissue stiffness.

1. Lamin-A and collagen levels scale with tissue stiffness

(A) Quantitative proteomics of multiple human and mouse tissues and cells revealed scaling with $E$ of the stoichiometry of lamin-A to lamin-B through mass spectrometry quantification of a pan-lamin peptide. Nuclei with abundant lamin-A are stiff. Cultured cells showed the same trend as their primary counterparts. Mass spectrometry trends were validated by immuno staining. (B) Lamin-A/B ratios vary weakly with $E$, while lamin-B2 is constant on average. (C) Collogen-I contents scale strongly with $E$. (D) Microscope assay of an ASH cell nucleus expressing GFP-lamin-A shows extension of the lamina with time. (E) The response of the lamina can be considered as a combination of elastic and viscous components, with an elongation response time of ~ 5 µs. Microscopy observation over seconds-to-minutes timescales in cells with different E-A ratios.

2) Stress affects lamin-A conformation, phosphorylation and level

(A) ASH nuclei imaged following shear stress applied in the z-axis. Greater lamin-A expression confers mechanical stability to the nucleus, limiting disruption of chromatin. (B) A cysteine-rich nuclear label was added to nuclei during shear stress. Proteins were then solubilized and the extent of ligation quantified by mass spectrometry. The Ig-like domain of lamin-A has a cysteine-rich domain, Cys522, that is buried in the core of the nucleosome. Ligation of Cys522 in the tail of lamin-A did not change with stress.

3) Matrix and lamin-A level amplify decisions of cell fate

(A) Promoter-reporter construct for LMNA is annotated with six predicted binding sites of transcription factors in matrix and RNA pathway, and a deletion construct (Δ-LMNA) lacking four RA target element binding sites. (B) LMNA message is reduced with protein across tissue. (C) Antibodies (A) and agonist (RA) increase matrix elasticity and expression of a lamin-A reporter construct. (D) LMNA reporter activity significantly increases in MSCs grown on soft (0.3 kPa) vs. stiff (40 kPa) substratum.

4) Regulation of lamin-A

(A) Matrix elasticity directs stem cell differentiation, which is enhanced by lamin-A. (B) Overexpression (OE) of lamin-A in MSCs in combination with stiff matrix and an oasis-inducing media maximizes osteogenesis. Soft matrix suppressed osteogenesis in parallel cultures with no apparent effect of overexpression. (C) Alkaline phosphatase (ALP) staining with or without a week of exogenous lecithinogen signal, together with the fraction of cells with staining. (D) Partial knockdown (KD) of lamin-A with si-LMNA in combination with soft matrix (0.3 kPa) and an adipogenic matrix maximizes adipogenesis. Stiff matrix (40 kPa) stimulates osteogenesis in parallel cultures with no significant effect of knockdown. Knockdown of lamin-A was 35% of control or siRNA-5′ (E) Adipogenesis in MSCs on plastic coated with cells with oil droplets (phase contrast microscopy) was increased by overexpression but not by knockdown.

5) Regulation by lamin-A and matrix (RARG, SRF and YAP1)

(A) Confluent images of RARG in MSCs. Nuclear mid-sections showed cytoplasmic RARG on soft matrix and increasing localization of RARG to the nuclear periphery with increasing lamin-A levels. Nuclear-to-cytoplasmic ratio of RARG scale with matrix elasticity and was directly affected by lamin-A levels. (B) Correlation between protein and transcript changes as lamin-A is knocked down in MSCs. 485 proteins were quantified and 30% of them were significantly changed (P < 0.005). (C) Confluent microscopy of YAP1 in MSCs. Plot shows 4-fold increase in nuclear to cytoplasmic ratio of YAP1 with increasing matrix stiffness, except that lamin-A over-expression decreases nuclear YAP1.

6) A feedback-based gene circuit for lamin-A regulation

(A) Gene circuit connecting matrix stiffness to osteogenesis. LMNA protein levels are regulated by a stress-sensitive phosphorylation mechanism and feeds back into LMNA transcript through interaction with RARG, possibly through an intermediary, and can be perturbed with antagonists (AGN) or agonists (RA). LMNA protein also influences location of YAP1 (through a possible intermediary, and is a signal cascade, ligand, and YAP1 regulates RARG through interaction with nuclear activator. A simple model was generated based on this circuit: time-evolution of LMNA mRNA level is dependent on the LMNA protein level, while the protein level itself is regulated by a tension dependent degradation. The LMNA protein signal then feeds back to the matrix, which regulates protein turnover. Trajectories of lamin-A and RARG message and protein as the model converges from a range of initial conditions to a steady state solution appropriate to the tension. (B) Setting the lamin-A expression threshold coefficient, h, to be proportional to (Tension)$^{-1}$ allows the model to generate steady-state lamin-A scaling with tension consistent with experiment.