Rapid and Sustained Changes in Nuclear Architecture and Mechanics in Mesenchymal Stem Cells in Response to Dynamic Stretch

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Introduction

Mechanical cues direct lineage-specification of mesenchymal stem cells (MSCs)[1,2] and regulate gene expression and cell function via histone modification.[3] Chromatin condensation, often mediated by histone methylation, leads to an overall silencing of transcription, while preserving the activity of lineage-specific genes in less condensed (euchromatic) regions.[4] In previous work, we showed that dynamic loading (DL) could evoke remodeling of the nucleus, involving Lamin A/C reorganization and changes in heterochromatin, more rapidly than addition of soluble differentiation factors.[5] However, the mechanisms by which physical cues are translated to MSC lineage commitment remains unclear. In the current study, we interrogated the time scales over which DL regulates chromatin condensation, determined whether such changes in chromatin condensation altered nuclear mechanics, and the duration over which these changes were "imprinted" on the nucleus to establish a mechanical "memory." Further, we identified ATP as a key signaling molecule, and several mechanically regulated genes that may be responsible for stabilizing nuclei in the condensed state with mechanical perturbation.

Methods

Aligned poly(ε-caprolactone) nanofibrous scaffolds were fabricated via electrospinning.[2] Bovine bone marrow derived MSCs (2×10^5 cells) were seeded onto scaffolds (5×60 mm^2) and samples were dynamically stretched (3%, 1Hz)[2] for 4 different durations (short term: 150 or 600s sec, long term: 1 or 3 hour) in a chemically defined media (CM). Another set of constructs was pretreated with 10μM of the Rho kinase inhibitor, Y27632 for 1 hour (Y27) to block acto-myosin contractility. A chromatin condensation parameter (CCP) describing internal nuclear structure was calculated for individual nuclei stained with DAPI using a gradient-based Sobel edge detection algorithm.[6] To measure the permanency in nuclear condensation, samples were cultured for up to 18 hours after loading. At set time points, the degree of nuclear deformation (defined by change in nuclear aspect ratio (NAR)) was determined for statically stretched samples.[7] To investigate ATP as a potential mediator of chromatin remodeling, 5 UN of Apyrase (AP, Sigma, an extracellular ATP diphosphohydrolase) was added 30mins before loading. To determine the factors involved in mechanical memory, a further set of constructs was dynamically loaded for 3 hours/day, returned to free swelling culture for 48hours, and then subjected to another round of loading for 3 hours. CCP, NAR, and real time RT-PCR for expression of genes associated with chromatin remodeling (normalized to GAPDH) were assessed through 48hours after loading. Statistical analysis was performed by ANOVA with Fisher's post-hoc tests.

Results

Chromatin condensation (increased CCP) was evident in nuclei after just 600s of dynamic loading (Figure 1a,b). Blockade of contractility abrogated load induced change in CCP at early times (Figure 1a,b). Chromatin condensation persisted for durations that depended on the length of the original stimulation: after 150s or 600s of loading CCP gradually decreased to base line levels by 3 hours, while with 1 hour of loading chromatin relaxation was slower (Figure 1c). Interestingly, with 3 hours of DL, chromatin condensation did not decrease, but rather increased through an 18-hour window of observation (Figure 1c). Under these conditions, nuclei did not deform either 600s or 1 hr after cessation of loading (for 600s) compared to unloaded controls (Figure 1d). However, by 3 hours after loading, NAR once again increased with stretch, matching controls (Figure 1d). AP treatment blocked increases in CCP with 150s or 600s of loading, suggesting that ATP may mediate increases in CCP (Figure 2). Interestingly, a 2nd loading event (applied 48 hours after the first, Figure 3c) resulted in more chromatin condensation than during the 1st loading event (Figure 3a). Moreover, this condensation was sustained for a longer time period with the 2nd loading than with the 1st loading. Further, while nuclear deformation was apparent 48 hours after the first loading when cells were stretched, nuclei did not deform when static stretch was applied 48h after the 2nd loading

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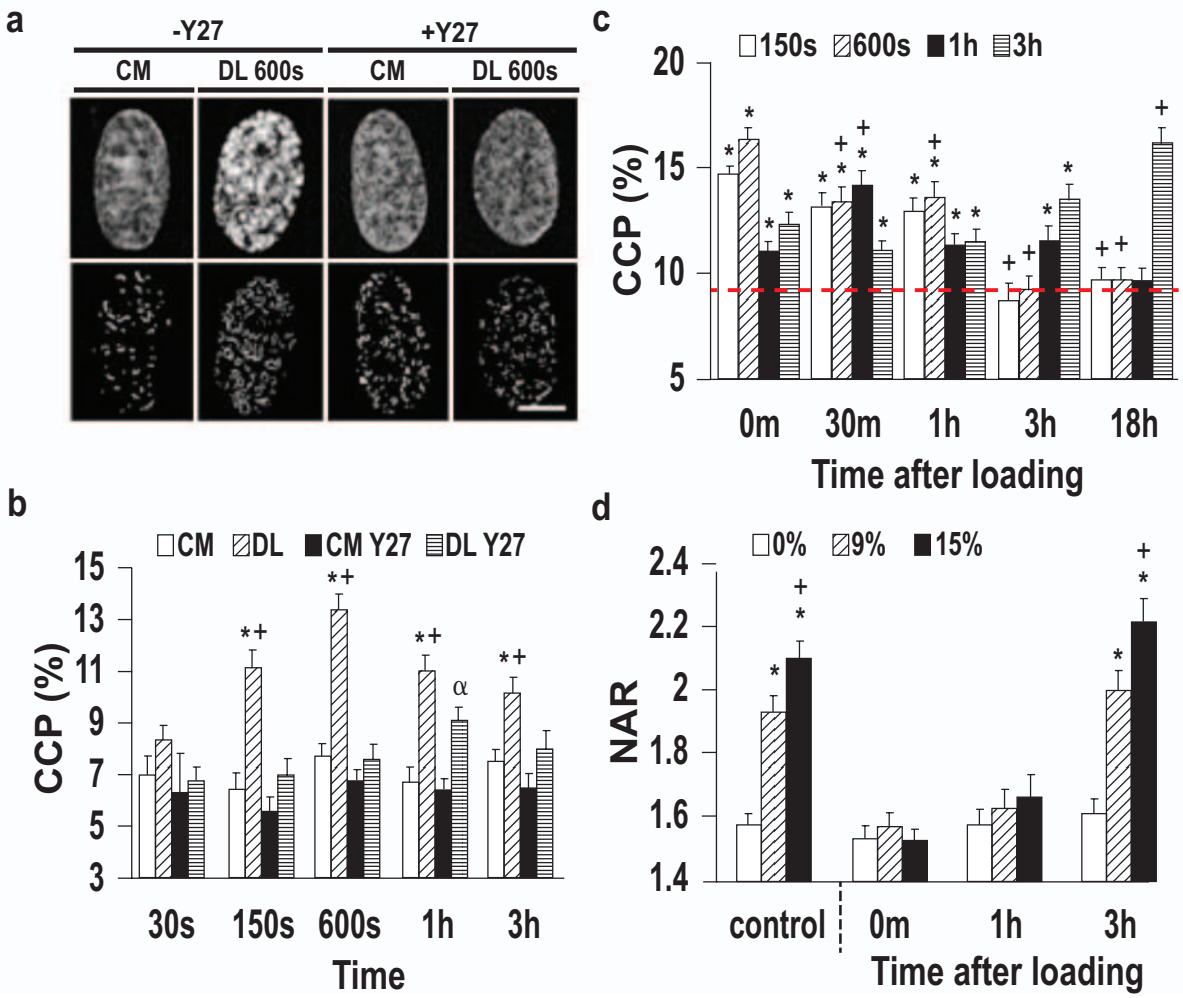

Figure 1. (a) Representative DAPI stained nuclei (top row) and corresponding edge detection (bottom row) with treatments (DL or Y27, bar = 3 μm). (b) CCP with treatments (DL or Y27, n=30, *: $p<0.05$ vs. CM, +: $p<0.05$ vs. Y27, α: $p<0.05$ vs. CM Y27, mean±SEM). (c) Permanency of CCP induced by DL (dashed line: CM condition, n=30, *: $p<0.05$ vs. CM, +: p40, *: $p<0.05$ vs. 0%, +: $p<0.05$ vs. 9%, mean±SEM).

event (not shown). Gene expression of SMC1A, a subunit of the cohesion complex that mediates chromatin condensation, was up-regulated as a consequence of both loading events, and was sustained at this higher level during rest periods between loading (Figure 3c). The 2nd loading event resulted in a greater increase in AGG gene expression than with the 1st loading event, and this level continued to increase after the 2nd loading (Figure 3d).

Discussion

In this study, we demonstrated that, in the absence of exogenous differentiation factors, short term loading of MSCs results in rapid chromatin condensation. This finding suggests that dynamic mechanical inputs can induce a rapid change in nuclear structure. The load induced condensation was abrogated with addition of a Rho kinase inhibitor, suggesting the requirement of a patent acto-myosin contractility apparatus.[8] In addition, removal of extracellular ATP blocked increases in CCP, consistent with the notion that mechanical forces can induce extracellular ATP release.[9] Interestingly, chromatin condensation persisted for durations that depended on the

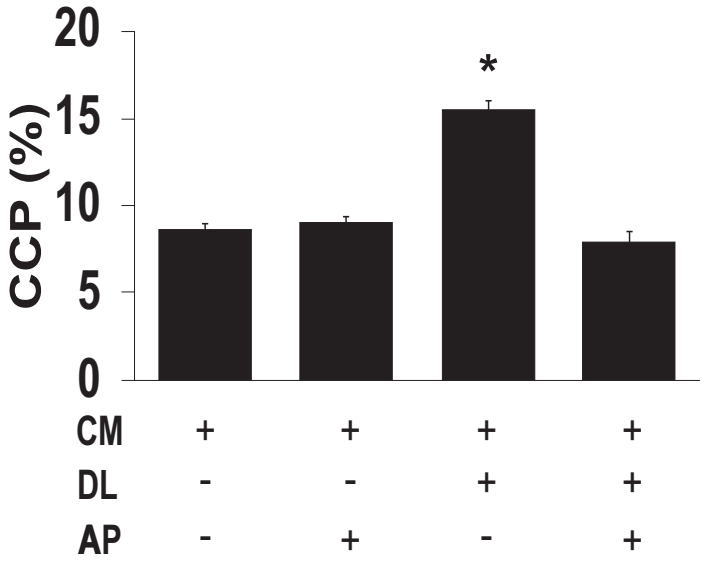

Figure 2. CCP with treatments (600s of DL or AP, n=30, *: $p<0.05$ vs. CM only).

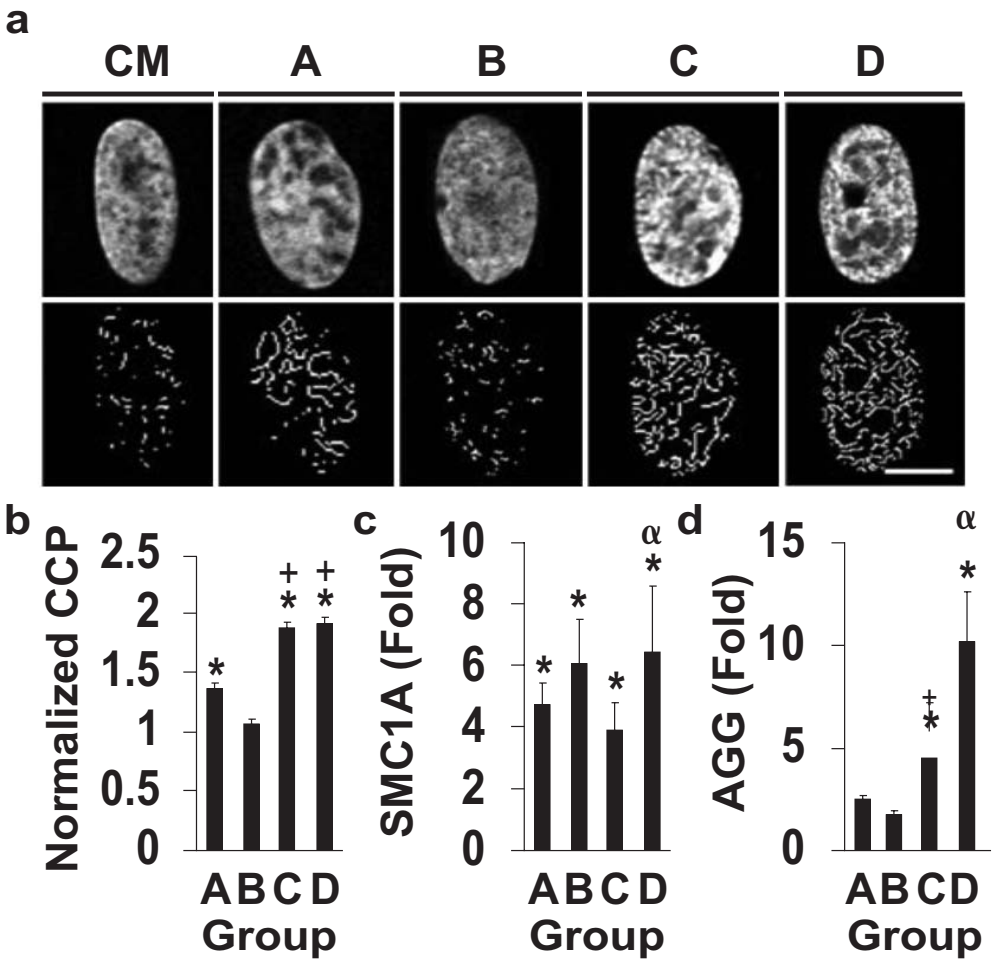

Figure 3. (a) Representative DAPI stained nuclei (top row) and corresponding edge detection (bottom row) (A: 1st loading, B: 48 hours after the 1st loading, C: 2nd loading, D: 48 hours after the 2nd loading, bar = 3 μm). (b) CCP normalized to CM condition (n=200 from 4 replicates, *: $p<0.05$ vs. CM condition , +: $p<0.05$ vs. B, mean ± SEM). (c) SMC1A and (d) AGG gene expression (n=3, *: $p<0.05$ vs. CM, +: $p<0.05$ vs. B, α: $p<0.05$ vs. C, mean±SD).

length of the original stimulation, and altered the manner in which nuclei deformed in response to static stretch. More interestingly, increasing the number of loading cycles increased the magnitude of chromatin condensation, and up-regulated expression of genes associated with chromatin movement (SMC1A) and ECM expression. This finding suggests that MSCs might establish a mechanical memory, encoded in structural changes in the nucleus that sensitizes these cells to future mechanical loading events.

Significance

Mechanical cues play an important role in directing MSC lineage specification. However, the mechanism is poorly understood, and the role of nuclear structure and mechanics has not yet been fully elucidated. Here, we show that mechanical stimulation results in a rapid remodeling of nuclear chromatin, that loading duration influences the persistence of these changes, and that loading establishes a mechanical "memory," priming cells to respond to further mechanical perturbation.

Acknowledgments

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