Cytoskeletal Tension is Required for Dynamic Tensile Loading Induced Alterations in Mesenchymal Stem Cell Shape and Nuclear Connectivity

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Introduction

Marrow derived mesenchymal stem cells (MSCs) show promise for orthopaedic tissue engineering applications. MSC differentiation is influenced by micro-environmental factors, some of which regulate cell and nuclear shape.[1] Aligned electrospun scaffolds provide a fibrous template, which mimics fibrocartilagenous tissue structure and allows for application of physiologic mechanical cues.[2] Cytoskeletal tension, regulated by rho kinase (ROCK), plays an important role in MSC interpretation of these environmental cues,[1] and is significantly increased with TGF-β3 induced differentiation.[3] These cues can be transmitted through the contractile actin cytoskeleton, and the LINC complex (linker of the nucleus and cytoskeleton), to the nucleus. Nesprin 1 giant is a large (~MDa) LINC complex protein that plays a role in differentiation and mechanotransduction for a variety of cell types.[4,5] Nesprin connections to the tensed actin cytoskeleton may provide a direct nuclear mechanotransduction mechanism in MSCs. However, the extent to which cytoskeletal tension and connectivity to the nucleus, contributes to MSC differentiation and mechanotransduction is currently unknown. This study aims to determine the role of MSC cytoskeletal tension in the transmission of mechanical signals to the nucleus, and how these mechanical signals impact nuclear connectivity and differentiation.

Methods

Static Stretch: Aligned nanofibrous scaffolds were formed by electrospinning poly(ε-caprolactone) onto a rotating mandrel.[2] Fibronectin coated scaffolds (65 x 5 mm^2) were seeded on each side with 2x10^5 bovine MSCs. Seeded constructs were cultured in a chemically defined media (CM) with or without (+/-) TGFβ3 (10ng/ml) for 7 days. Nuclei were stained with Hoechst (5μg/ml) in DMEM for 20 min and statically stretched (3% increments to 15% strain) using a microscope-mounted device with or without 1 hour treatment with the ROCK inhibitor Y27632 (10μM, Calbiochem). The ratio of the nuclear principal lengths (nuclear aspect ratio, NAR) was calculated at each strain level to quantify nuclear deformation.

Dynamic Stretch: Seeded constructs were pre-cultured for 2 days and then dynamically loaded (DL; 1Hz; 3% strain, 6 hours) in CM(-) for 2 days with or without Y27632 (10μM), which was added 1 hour before the start of loading each day. On day 4, samples were fixed in 4% PFA and stained for F-actin (phalloidin) for quantification of the cellular aspect ratio (CAR) using image J. Additionally, mRNA and whole cell protein was isolated (n=3/grp). qPCR was performed to determine expression of aggrecan, scleraxis, and nesprin 1 giant. Cell lysates (n=3/grp) were separated by column filtration into >1MDa and <1MDa fractions for dot blot analysis of the large splice variant of nesprin 1 (giant, ~1MDa). Western blots were performed for β-actin normalization. One-way and two-way ANOVA with Tukey's post hoc was used to determine significance between groups ($p<0.05$).

Results

Static stretch experiments showed that undifferentiated MSCs displayed significantly more nuclear deformation at 12 and 15% strain compared to MSCs exposed to TGF-β3 (Fig1A, n=40/grp). This nuclear deformation was abrogated by the addition of the ROCK inhibitor Y27632 (Fig1A), indicating that cytoskeletal tension is necessary for this strain transfer to occur. With dynamic stretch over 2 days, there was a significant increase in cellular elongation, as indicated by an increase in the cellular aspect ratio (n=103/grp) (Fig1B,C). ROCK inhibition resulted in rounder cells for all groups and prevented cellular elongation with dynamic loading (Fig1B,C). qPCR showed that CM(+) and dynamic loading (DL) increased expression of the chondrogenic marker aggrecan (not shown), the tenogenic marker scleraxis (Fig2A), and nesprin 1 giant (Fig2B). ROCK inhibition completely prevented the increase in scleraxis expression with dynamic loading (Fig1D), and partially (but not significantly) blocked the increase in nesprin 1 giant expression (Fig2A,B). Analysis of nesprin 1 giant protein levels (by dot blot) showed a significant increase in nesprin 1

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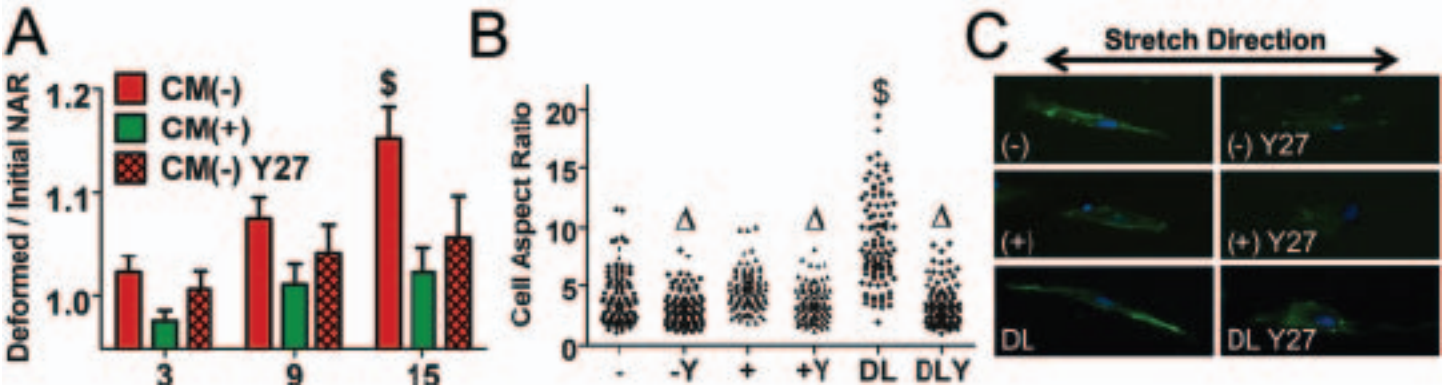

Figure 1A-B. Nuclear deformation (A) with static stretch in 3% increments to 15% strain (mean +/− SEM). $ = p<0.05 compared to both other groups (+/− indicates with or without TGF-β3). Quantification of changes in cell aspect ratio (B) after dynamic loading (DL) with or with out Y27632 (Y), and representative images of cells stained for actin/nuclei (green/blue). Δ = p<0.05 compared to non-Y control.

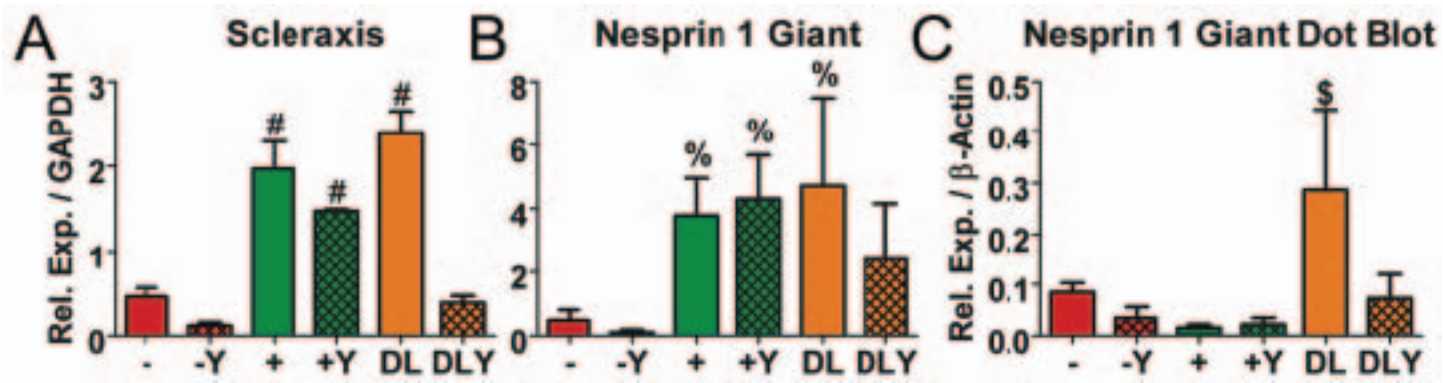

Figure 2A-B. Scleraxis expression (A) and nesprin 1 giant expression (B) following 2 days of DL (mean +/− SD). qPCR data is normalized to GAPDH expression. Quantification of dot blot for nesprin 1 giant normalized to β-actin (C). $ = p<0.05 compared to all groups; #=p<0.05 compared to −, −Y and DLY; %=p<0.05 compared to − and −Y.

giant with dynamic loading, which was prevented by ROCK inhibition (Fig2C).

Discussion

The cellular microenvironment regulates cell shape and MSC fate decisions in a manner that is dependent on the contractility of the actin cytoskeleton.[1] Additionally, this microenvironment can alter the cellular response to mechanical stimulation.[6] Transmission of these mechanical signals from the microenvironment to the nucleus, through the LINC complex and the actin cytoskeleton, provides one mechanism by which cells might respond to mechanical cues. In this study, loss of cytoskeletal tension (by ROCK inhibition) prevented nuclear deformation and dynamic tensile load induced changes in cellular morphology and gene expression. This included prevention of the changes in nesprin 1 giant, a component of the LINC complex that directly connects F-actin to the nuclear envelope, indicating a potential role for cytoskeletal tension and nuclear deformation in regulating the LINC complex. Interestingly, the changes in nesprin 1 giant protein expression in response to load were distinct from TGF-β3 induced differentiation. These differences in nuclear connectivity (through nesprin 1 giant) may result in an altered cellular interpretation of mechanical stimuli. For example, increased nesprin 1 giant may lead to an altered stress distribution at the nuclear envelope, thus influencing activation of nuclear mechanosensative proteins, and providing a potential feedback mechanism by which cells tune there sensitivity to mechanical stimulation. Further, this may be important for understanding the distinction between load-induced and biochemically induced differentiation of MSCs.

Significance

An understanding of how MSCs sense and respond to mechanical forces will be important for their effective use in tissue engineering. In this study, the influence of altered cellular contractility on MSC nuclear deformation, mechanotransduction and differentiation was investigated.

Acknowledgements

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