Matrix Elasticity is sensed with Non-Muscle Myosin II and Directs Stem Cell Lineage Specification

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Statement of Purpose: Many anchorage-dependent, contractile cell types respond to substrate elasticity, i.e. – Young's Modulus E, as sensitively as more well studied soluble or immobilized ligands, yet mechanisms by which cell-generated forces induce outside-in mechanical signals have been far less explored [1, 2]. Pluripotent cells, such as mesenchymal stem cells (MSCs), differentiate into a variety of connective tissues, including, nerve, muscle, and bone, that all have dramatically different microenvironments. Specifically, MSC lineage specification has only been studied via chemical stimuli [3, 4] or changes in cell shape and proliferation [5], yet current stem cell therapies [6] in fibrotic tissue compared to normal tissue [7] are limited by mechanical properties of the surrounding rigid matrix [8]. Given the limited capacity for MSC differentiation in an extremely stiff matrix, we explored whether a range of substrate stiffnesses could drive MSC lineage specification.

Results:

Cell Morphology Suggests Lineage Commitment

On soft, collagen-coated gels that mimic the elasticity of brain tissue ($E_{brain} \approx$ 0.1-1 kPa), the vast majority of MSCs exhibit a branched morphology (Fig. 1A), with branching densities of MSCs approaching those of primary neurons on matrigel-coated gels [9]. In contrast, on ten-fold stiffer substrates that mimic the elasticity of striated muscle ($E_{muscle} \approx$ 8-17 kPa), MSCs develop myoblast-like, spindle shapes. Considerably stiffer substrates ($E_{Osteoblast}$ > 30 kPa) that seem reasonable mimics of collagenous bone yield MSCs that are more polygonal and similar in shape to osteoblasts. Quantitative analysis of cell shapes (not shown) demonstrates that variations in morphology are about the same for MSCs as they are for differentiated neural and myogenic cells.

Cytoskeletal and Transcription Protein Expression Suggests Lineage Commitment

A majority of cells on the softest, neurogenic matrices express the intermediate filament proteins phosphorylated neurofilament heavy chain (P-NFH; arrowheads, Fig. 1B) and β3-tubulin. Proteins are visible in long, branched extensions and are poorly expressed, if at all, in cells on stiffer gels. On substrates near $E_{Skeletal}$ (8–17 kPa), MSCs preferentially expressed Myogenic Differentiation Factor 1 (MyoD1; arrow), a myogenic transcriptional marker; cells on softer and stiffer gels did not express MyoD1 in a similar mechanosensitive manner as muscle striation [1]. Only while on substrates near $E_{Osteoblast}$ did MSCs (arrow) express Core Binding Factor α1 (CBFα1; arrow), a nuclear-localized osteogenic transcriptional protein, corresponding to their polygonal morphology.

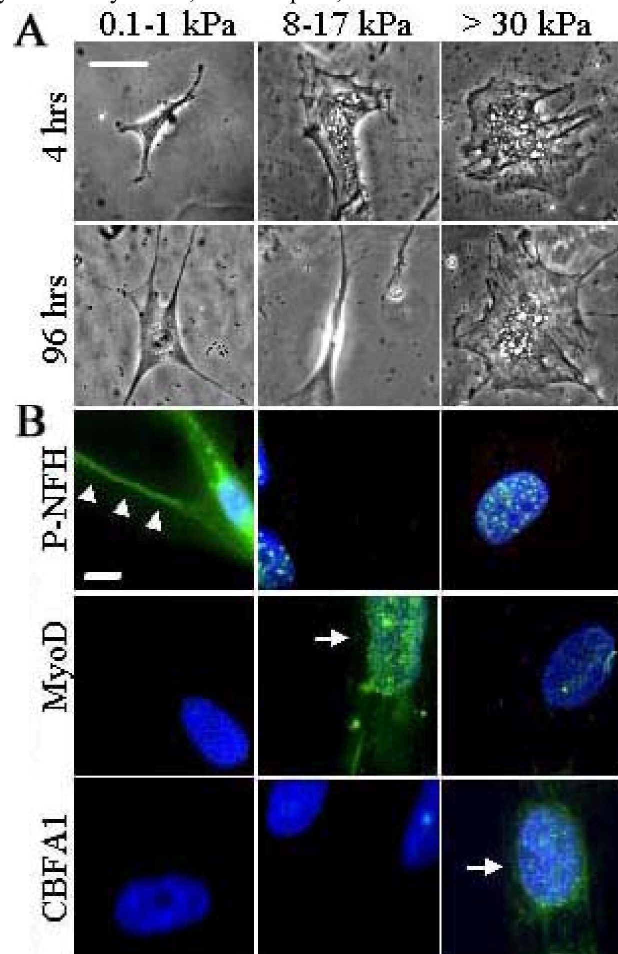

Figure 1: Elastic modulus controls morphology and lineage commitment. White arrows and arrowheads indicate positive staining. Scale bars are 50 and 20μm for A and B, respectively.

MSCs couple NMM II expression to matrix stiffness

Select myosins appear more matrix sensitive than others, based on clustering of microarray data by variation with E. Non-muscle myosin II (NMM II) western blots shows matrix elasticity sensitivity, and blebbistatin inhibition blocks its expression/function, while preventing upregulation of MyoD, desmin, and CBFα1. Immunoflorescence indicates diffuse NMM II staining for MSCs near E_{brain} and stress fibers for $E_{Osteoblast}$, while on E_{muscle}–gels myosin striations emerge with a periodic spacing similar to age-matched myoblasts. Data indicates that this is due to Rho GTPase changes.

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