

# Fibroblasts within Mouse Subcutaneous Fascia Respond to Tissue Stretch with $\alpha$ -Smooth Muscle Actin Redistribution and Changes in Nuclear Shape

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**BACKGROUND** Direct transmission of forces through the cytoplasmic and nuclear cytoskeleton via changes in cell and nuclear shape has been proposed as a source of specific coupling between tissue mechanical forces and genome. This study aims to investigate the effect of tissue stretch on 1) nuclear shape and 2) the spatial organization of  $\alpha$ -Smooth Muscle Actin ( $\alpha$ -SMA) in the nucleus of whole mouse subcutaneous tissue fibroblasts. Nuclear morphology and a dynamic response to tissue stretch may be closely associated with cytoskeletal remodeling and contractility which could possibly aid in coordinating chromatin remodeling and gene expression with the cell's ambient mechanical environment.

**METHODS** Subcutaneous connective tissue was harvested after death from the abdomen of C57BL/6 male mice. Whole skin flaps (8 cm x 3 cm) containing dermis, subcutaneous muscle and subcutaneous tissue were dissected away from the abdominal wall musculature, excised, placed between stainless steel grips, and submerged in HEPES-physiological saline solution. Skin flaps were stretched by elongating the tissue (20% strain) for 30 minutes or incubated without stretch. Following tissue fixation and sample preparation, immunohistochemistry was performed for the detection of  $\alpha$ -SMA using a mouse monoclonal anti- $\alpha$ -SMA primary antibody, and the tissue was counterstained with nucleic acid marker DAPI. 326 cells were imaged by confocal microscopy and evaluated by both quantitative and subjective measures of image organization.

**RESULTS** In unstretched tissue,  $\alpha$ -SMA staining was located within deep nuclear invaginations containing cytoplasm. In stretched tissue the  $\alpha$ -SMA staining was more homogenously distributed within the nuclear domain and fewer visible invaginations were seen. Furthermore, the overall nuclear shape changed in response to mechanical tissue stretch (or lack thereof). While the nuclear volume remained relatively unchanged in the nuclei of unstretched vs. stretched tissue (Mean  $\pm$  SE 327.47  $\pm$  13.91 and 366.68  $\pm$  12.84 respectively), the nuclear cross-sectional area decreased for the nuclei of unstretched tissue when compared with those of stretched tissue (91.83  $\pm$  3.16 and Mean  $\pm$  SE 113.74  $\pm$  4.75), while the nuclear thickness increased (11.04  $\pm$  0.25 unstretched tissue and Mean  $\pm$  SE 9.9  $\pm$  0.36 stretched tissue). These data suggest that the nuclei change shape (i.e. become broader and flatter in the plane of the tissue) with stretch. A blind observer subjectively measured this observation by rating each nucleus on a scale of 0 to 3 (0=broad/flat nuclear shape and 3=convoluted nuclear shape). The subjective ratings of nuclei in unstretched vs. stretched tissue were Mean  $\pm$  SE 1.48  $\pm$  0.08 and 0.86  $\pm$  0.13 respectively. We are currently developing an objective outcome measure of nuclear convexity and anticipate having completed this new analysis by October 2007.

**CONCLUSION** Our preliminary results show that tissue stretch causes a change in nuclear shape and a redistribution of  $\alpha$ -SMA within the cell. These findings may have important implications for our understanding of mechanical signal transduction from the cell periphery to the nucleus.

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