The Impact of Matrix Stiffness and O-GlcNAcylation on YAP Nuclear Localization and Matrix Deposition in Mesenchymal Stem Cells

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Introduction

Mechanotransduction, the process in which mechanical stimuli are converted into biochemical signals, can influence cellular behavior. In mesenchymal stem cell (MSCs), yes-associated protein, YAP, becomes increasingly localized to the nucleus in stiff microenvironments, impacting lineage specification and cellular activities, including matrix gene expression[1]. While the mechanical mechanisms by which YAP nuclear translocation occurs is increasingly well defined, biochemical modifiers of YAP in the cytosol also impact YAP baseline levels, bioavailability, and activity. Recent findings suggest that the transfer of an O-linked β-*N*-acetylglucosamine (O-GlcNAc) group to a specific serine or threonine on YAP can control its nuclear localization[1]. Based on this, we hypothesized that modification of YAP by O-GlcNAc transferase (OGT) would influence nuclear translocation in a mechanobiologic setting. In addition, we queried the impact of substrate stiffness on nascent extracellular matrix (ECM) deposition, using functional non-canonical amino acid labeling (FUNCAT)[2]. The timing and amount ECM deposition defines tissue maturation and structure-function relationships, and is uniquely tuned to enable musculoskeletal tissue function. While matrix stiffness increases mechanobiologic signaling (e.g., YAP nuclear localization) in MSCs, the link between mechanosensing and nascent matrix production has not yet been explored. To that end, we utilized norbornene-modified hyaluronic acid (NorHA) hydrogels to evaluate substrate stiffness and ECM formation.

Methods

Hydrogel Fabrication

Fibronectin-coated polyacrylamide (PA) gels were produced as in[3] at stiffnesses of 5, 15, or 55kPa. RGD-modified NorHA hydrogels were produced as in[4] to a stiffness of 5kPa or 15kPa.

Cell culture

For YAP O-GlcNAcylation studies, juvenile bovine bone marrow MSCs (bMSCs) and NIH3T3 (3T3) cells were cultured in DMEM with 10% FBS. For nascent ECM studies, hMSCS were cultured in DMEM (without methionine) supplemented with the methionine analog azidohomoalanine (AHA) to mark nascent proteins. For this, cells were seeded on NorHA gels for 24 hours before fixing.

Pharmacological inhibition

OGT was inhibited with OSMI-1 (at 50mM). Cells were resuspended in media with or without OSMI-1, and seeded on PA hydrogels overnight before fixing. Cellular contractility was inhibited by Y-27632 (at 10uM) and increased with LPA (at 50uM).

Immunocytochemistry and AHA labeling

To visualize YAP, cells were fixed in 4% PFA, permeabilized, incubated in primary antibody overnight, and nuclei were stained with DAPI. To identify nascent matrix, DBCO-488 was used to label all proteins containing AHA. A membrane stain was used to create a mask for subsequent analysis. In order to identify only exogenous proteins, labeling was performed on live cells prior to fixation.

Image and statistical analysis

The ratio of nuclear to cytoplasmic (N:C) YAP was determined by tracing cell and nuclear boundaries and calculating fluorescence intensity with Image J. Nascent matrix was quantified using the cell membrane stain as a mask to eliminate cell surface receptors; this was subtracted from the ECM stain in ImageJ. Resulting images were quantified using the 3D objects plugin in ImageJ to obtain ECM volume, and was normalized to cell volume. Statistical analysis was performed by one-way ANOVA with Tukey's post-hoc tests.

Results

In 3T3 cells and bMSCs, YAP N:C ratios increased with increasing substrate stiffness (Figure 1A,B). In bMSCs and 3T3 cells on 15kPa and 55kPa PA gels, YAP N:C ratios decreased in the presence of OSMI-1, to levels matching that of a 5kPa gel (Figure 1A,B). Cells on glass showed a partial (but significant) reduction in YAP N:C ratio in bMSCs with OSMI-1 treatment. Qualitatively,

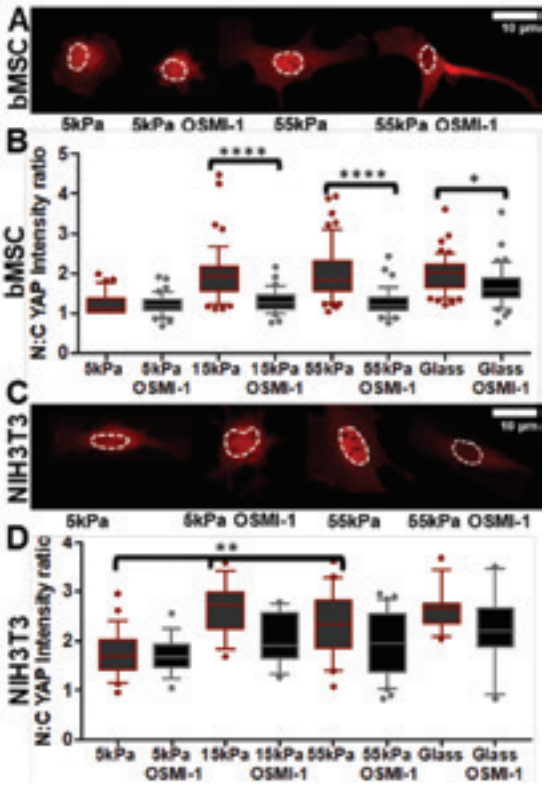

Figure 1. OSMI-1 decreases N:C YAP in bMSCs and 3T3 cells. Images of YAP (red) in **[A]** bMSCs and **[C]** 3T3s at varying stiffnesses. Nuclei depicted with dotted line. Box and whisker plots for **[B]** bMSCs (N = 3 replicates, n = 39-53 cells) and **[D]** 3T3 cells (N = 2 replicates, n = 14-30 cells).

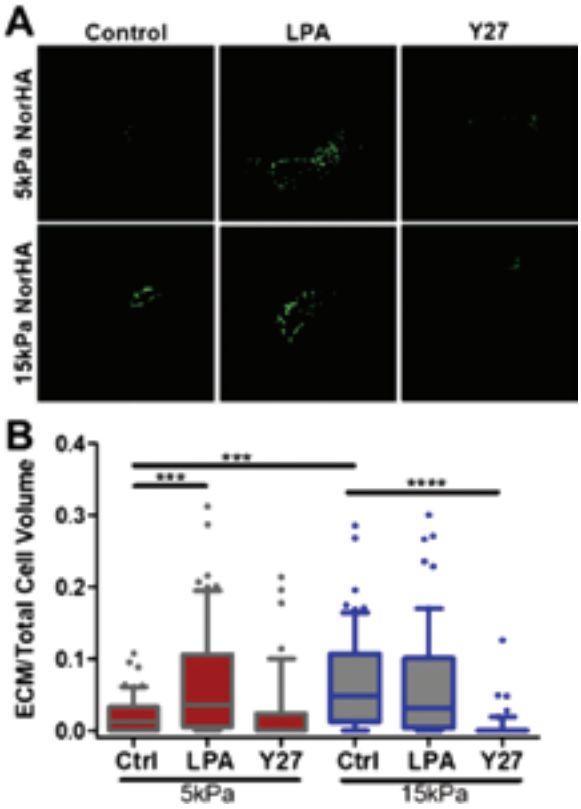

Figure 2. Substrate stiffness and contractility increase nascent ECM production. AHA staining (green) in hMSCs on gels of 5kPa or 15kPa, with or without contractility agonist (LPA) or antagonist (Y27) **[A].** Quantification of AHA staining (ECM/total cell volume) **[B].** Box and whisker plots for **[B]** (N = 3 replicates, n = 45-63 cells).

total YAP levels and cell spreading were not changed with OGT inhibition. However, preliminary findings suggested that inhibition of OGT in hMSCs on NorHA gels decreased the number of focal adhesions, compared to untreated cells. This would suggest a decrease in traction force exerted by cells with OGT inhibition. With regards to nascent ECM, hMSCs on 5kPa NorHA gels produced little matrix, while hMSCs on 15kPa NorHA gels produced *significantly* more matrix after 1 day (Figure 2A,B). Matrix production was abrogated on both stiffnesses with inhibition of cellular contractility with Y27 (Figure 2A, right panels). Conversely, increasing contractility with LPA on 5kPa NorHA gels increased ECM production to levels matching that of cells on 15kPa NorHA gels (Figure 2A,B). After 3 days of culture, however, no differences in nascent matrix accumulation were seen, with cells on both stiffnesses accumulating similar amounts (data not shown).

Discussion

Taken together, our results demonstrate that OGT is a powerful biochemical regulator of YAP, which can impact its nuclear localization under mechanically inductive conditions. Because glucose availability regulates O-GlcNAc[1], and native tissues have different levels of glucose availability, it could be that certain tissues mechanotransduce in a different manner based on metabolic mechanisms. Our results also show that cellular contractility directly regulates nascent matrix deposition and assembly. Biomaterials for tissue engineering have intrinsic mechanical and adhesive properties, and this may impact nascent matrix produced by cells. Future work will be investigate the downstream consequences of cell autonomous ECM production on MSC differentiation. Further, given that O-GlcNAcylation processes mediate modification of both YAP and extracellular proteins (which is necessary for their function) future work will identify possible crosstalk between the metabolic state of the cell and its mechanobiological state.

Significance

O-GlcNAcylation of YAP is an important metabolic modifier of cell mechano-transduction. The role of cellular contractility and baseline mechano-signaling on nascent matrix production is important for the design of biomaterials that may direct tissue formation by MSCs.

Acknowledgements

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References

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